

Transpiration efficiency over an annual cycle, leaf gas exchange and wood carbon isotope ratio of three tropical tree species

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Summary Variation in transpiration efficiency (TE) and its relationship with the stable carbon isotope ratio of wood was investigated in the saplings of three tropical tree species. Five individuals each of *Platymiscium pinnatum* (Jacq.) Dugand, *Swietenia macrophylla* King and *Tectona grandis* Linn. f. were grown individually in large (760 l) pots over 16 months in the Republic of Panama. Cumulative transpiration was determined by repeatedly weighing the pots with a pallet truck scale. Dry matter production was determined by destructive harvest. The TE, expressed as experiment-long dry matter production divided by cumulative water use, averaged 4.1, 4.3 and 2.9 g dry matter kg⁻¹ water for *P. pinnatum*, *S. macrophylla* and *T. grandis*, respectively. The TE of *T. grandis* was significantly lower than that of the other two species. Instantaneous measurements of the ratio of intercellular to ambient CO₂ partial pressures (c_i/c_a), taken near the end of the experiment, explained 66% of variation in TE. Stomatal conductance was lower in *S. macrophylla* than in *T. grandis*, whereas *P. pinnatum* had similar stomatal conductance to *T. grandis*, but with a higher photosynthetic rate. Thus, c_i/c_a and TE appeared to vary in response to both stomatal conductance and photosynthetic capacity. Stem-wood $\delta^{13}\text{C}$ varied over a relatively narrow range of just 2.2‰, but still explained 28% of variation in TE. The results suggest that leaf-level processes largely determined variation among the three tropical tree species in whole-plant water-use efficiency integrated over a full annual cycle.

Keywords: *Platymiscium pinnatum*, stem-wood $\delta^{13}\text{C}$, *Swietenia macrophylla*, *Tectona grandis*, water-use efficiency.

Introduction

Transpiration efficiency (TE) describes the rate of plant dry matter production for a given rate of plant water loss to the atmosphere. At the ecosystem scale, it is a measure of the coupling between the net primary production and the transfer of water from soil to the atmosphere through vegetation. Thus, variation in TE could have important implications for both carbon cycling and catchment water balance. For example, TE tends to increase with increasing atmospheric CO₂ concentration (Morison 1985, Eamus 1991, Farquhar 1997, Winter et al. 2001). If the increase in TE is not matched by a corresponding increase in productivity, the net result may be a decrease in canopy-scale transpiration. Such a response has been implicated in increased continental river runoff during the 20th century (Gedney et al. 2006).

Given the potential for TE to affect both the performance of individual plants and ecosystem-level processes, there has long been an interest in quantifying it (Woodward 1699, Lawes 1850, Briggs and Shantz 1914, Shantz and Piemeisel 1927, Fischer and Turner 1978, Bacon 2004). However, direct measurements of TE are not easily obtained for whole plants, because they require the quantification of cumulative transpiration and dry matter production over extended periods. In recent decades, portable gas-exchange systems have become readily available, which allow instantaneous measurements of leaf-level water-use efficiency under field conditions (Long et al. 1996). Extrapolating trends in such measurements provides one means of inferring variation in TE. Additionally, measurements of the carbon isotope ratio ($\delta^{13}\text{C}$) of plant organic material have frequently been employed to infer time-integrated variation in leaf-level water-use efficiency, after it was recognized that $\delta^{13}\text{C}$ correlates with the ratio of intercellular to ambient CO₂ partial pressures (c_i/c_a) in C₃ plants (Farquhar et al. 1982, Farquhar and Richards 1984). Measurement of $\delta^{13}\text{C}$ has the further advantage of analyses being performed retrospectively. For example, measurements of tree-ring

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$\delta^{13}\text{C}$ have been used to infer historic variation in TE from preindustrial times to the present day (Marshall and Monserud 1996, Duquesnay et al. 1998, Feng 1998).

Although the measurements of instantaneous c_i/c_a and $\delta^{13}\text{C}$ are frequently employed as indicators of variation in TE, there have been relatively few comparisons with direct measurements of TE, especially for multiple species over a full annual cycle. For woody tree species, comparisons have generally focused on seedlings smaller than about 100 g dry mass (Zhang and Marshall 1994, Guehl et al. 1995, Sun et al. 1996, Osório et al. 1998). Ontogenetic variation in canopy architecture or leaf gas-exchange characteristics may cause variation in TE as tree seedlings increase in size. Additionally, tree species may respond differently to seasonal variation in evaporative demand, such that TE integrated over an annual cycle may differ from that measured over sub-annual time periods.

In this study, we describe the measurements of TE in three tropical tree species over a 16-month period. Plants were grown in 760 l pots, and the plant dry mass increased by several kilograms during the course of the experiment. The three species studied, *Platymiscium pinnatum* (Jacq.) Dugand (Fabaceae), *Swietenia macrophylla* King (Meliaceae) and *Tectona grandis* Linn. f. (Verbenaceae), previously displayed high, intermediate and low TE, respectively, when grown in 38 l pots for several months (Cernusak et al. 2007a, 2008). Our objectives were (1) to test whether the pattern of TE among the species observed previously would be maintained in saplings grown in large pots over both wet and dry seasons and (2) to compare the sapling TE integrated over the full 16-month period with the measurements of instantaneous c_i/c_a and stem-wood $\delta^{13}\text{C}$.

Materials and methods

The experiment was conducted in Gamboa, Republic of Panama at the Santa Cruz Experimental Field Facility, Smithsonian Tropical Research Institute (9°07' N and 79°42' W). The site is ~ 28 m a.s.l. It has a mean annual temperature of 26 °C and a mean annual precipitation of 2100 mm. There is a pronounced dry season that typically lasts from the middle of December until the end of April. Detailed meteorological data for the site are available at http://striweb.si.edu/esp/physical_monitoring/downloads_intro.htm.

Five seedlings each of *P. pinnatum*, *S. macrophylla* and *T. grandis* were transplanted into 0.76 m³ pots, with one seedling in each pot. The seedlings had previously been growing in 4 l pots and were ~ 6 months old when transplanted. Each 0.76 m³ pot was filled with a homogeneous soil mixture, comprising 80% by volume dark brown topsoil and 20% by volume air-dried rice husks. The rice husks were added to improve soil structure and drainage. Additionally, 24 g controlled-release fertilizer (Osmocote-Plus, Scotts-Sierra, Maryville, OH) was added to each pot. The

fertilizer contained by weight 15% N, 9% P and 12% K, and had an estimated release time of 5–6 months. Before the seedlings were transplanted, the pots were saturated with water and allowed to drain for several days to establish pot weights at field capacity. The drain holes were then sealed and remained so for the duration of the experiment. The mean pot weight at field capacity was 878 ± 27 kg (mean ± 1 SD). Three control pots without plants were prepared using the same procedure. The pots were wrapped in reflective insulation to prevent excessive heating. A skirt of reflective insulation was wrapped around the base of each seedling and overlapped the top of the pot to prevent rain-water from entering the pot and to reduce evaporation from the soil surface. Control pots had a wooden dowel in place of the seedling stem. The pots were placed in an open area, ~ 50 m from the forest edge, with a distance of 3–4 m between the pots. This location was chosen to enable access to the pots by the pallet truck scale (described below) and to minimize micro-site variation among the pots. Initial dry mass of the transplanted seedlings was estimated by harvesting the representative individuals of each species. The estimated initial dry masses were 23, 17 and 44 g for *T. grandis*, *S. macrophylla* and *P. pinnatum*, respectively.

Cumulative transpiration of each plant was determined from March 2007 until July 2008 by repeatedly weighing each pot with a pallet truck scale (PW800, A and A Scales LLC, Prospect Park, NJ). The scale had 2500 kg capacity and 0.5 kg graduation. The pots were placed on polyethylene pallets, such that the scale could be easily installed under each pot (McCulloh et al. 2007). Known quantities of water were added weekly to each pot, with the rate of water addition adjusted based on the rate of water consumption in the previous weighing interval. The pots were initially weighed monthly, then weekly toward the end of the experiment, when the water consumption peaked. Throughout the experiment, we aimed to maintain pot water contents above two-thirds field capacity. The control pots were weighed at regular intervals to estimate evaporation from the soil surface, and the mean water loss of control pots was subtracted from that of pots with plants to estimate plant transpiration. Summed over the full experiment, water loss from the control pots was only about 1% of that from the pots with plants. The mean plant fresh mass at the conclusion of the experiment (19 ± 7 kg; mean ± 1 SD) was less than 1% of the mean cumulative plant water use (2206 ± 801 kg), and therefore no attempt was made to adjust water uptake calculations for increases in plant biomass during the experiment. About 1 week before harvest, the pots were weighed at dawn and dusk to calculate daytime transpiration. Nighttime water loss was less than the resolution of the balance.

Leaf gas exchange was measured on 2 and 3 July 2008 with a Li-6400 portable photosynthesis system (Li-Cor Inc.). Scaffolding was used to access the crowns of the saplings. Irradiance during gas-exchange measurements was 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, supplied by an artificial light

source (6400-02B LED, Li-Cor Inc., Lincoln, NE). The leaf temperature was 31.7 ± 0.3 °C (mean \pm 1 SD), and the leaf-to-air vapor pressure difference was 1.0 ± 0.1 kPa. Eight leaves were measured from the mid-canopy of each sapling. Measurements were logged 1–2 min after the leaves were placed in the cuvette, which was sufficient time for gas-exchange readings to stabilize. The flow rate of air through the cuvette was $500 \mu\text{mol s}^{-1}$. Measurements were taken between 09:00 and 13:00 local time, under overcast conditions.

Saplings were harvested in July 2008, 16 months after the initiation of the experiment. Leaf area of each sapling was measured with a leaf area meter (Li-3100, Li-Cor Inc.). Leaves, stems and roots were dried to constant mass at 70 °C in large drying ovens. Stem height and diameter were measured, and a wood disk was removed at 0.8 m above the soil surface to quantify wood density. The height of 0.8 m exceeded the seedling height at the initiation of the experiment, such that the wood disk was produced entirely during the experiment. Wood density was calculated as fresh volume divided by the dry mass of the wood disk. Bark was not included in the measurement. Dry wood disks were then ground to a fine powder in a Cyclotec 1093 sample mill with a 0.5 mm screen (FOSS, Eden Prairie, MN) for analyses of stable carbon isotope ratio. Leaf litter was collected throughout the experiment and dried to constant mass at 70 °C.

Stable carbon isotope ratios of stem-wood dry matter were measured in the Stable Isotope Laboratory at the Smithsonian Tropical Research Institute. Samples of ~ 2 mg were combusted in an elemental analyzer (CE Instruments, Milan, Italy) and swept by a helium carrier gas via a continuous flow interface into an isotope ratio mass spectrometer (Delta V, Thermo Fischer Scientific, Waltham, MA). Carbon isotope ratios have been expressed relative to the PeeDee Belemnite international standard. The precision of the analyses, based on the repeated measurements of a working standard during the sample run, was $\pm 0.1\text{‰}$ (SD, $n = 12$).

Variation among the three species in morphological and physiological parameters was analyzed using analysis of variance. Where significant variation was detected, pairwise comparisons among species were performed according to Tukey's method. Variation among species in relationships between continuous variables was analyzed using analysis of covariance. Least-squares linear regression was used to analyze general relationships between continuous variables across the full dataset. Statistical analyses were performed in Systat 11 (Systat Software, Chicago, IL).

Results

Variation over the course of the experiment in mean daily transpiration of the three species is shown in Figure 1. Mean values for *T. grandis* were highest, those for

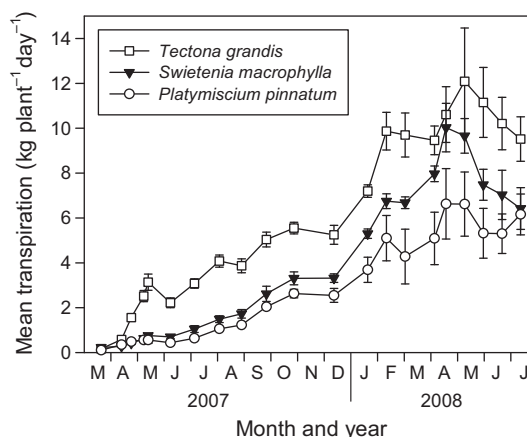


Figure 1. Mean daily transpiration of three tropical tree species over a 16-month period. Each data point is the average for five saplings during the interval preceding that point. Error bars represent one standard error.

P. pinnatum were lowest and those for *S. macrophylla* were intermediate. Mean daily transpiration for all three species peaked near April 2008. It then appeared to decline somewhat with the arrival of wet season conditions in May and June 2008 (Figure 1).

Morphological and physiological parameters for the saplings at the conclusion of the experiment are detailed in Table 1. Final dry mass of saplings ranged from 3.9 to 11.3 kg across all three species. Mean values did not vary significantly among species (Table 1). Final leaf area ranged from 4.4 to 15.1 m² and did not vary significantly among species. Mean stem height was highest in *T. grandis*, whereas mean stem diameter was highest in *S. macrophylla*. On the other hand, wood density was highest in *P. pinnatum*; *S. macrophylla* had a lower root/shoot ratio than either *T. grandis* or *P. pinnatum*. Mean relative growth rate over the full experiment was lower in *P. pinnatum* than in *T. grandis* or *S. macrophylla* (Table 1). This resulted from the higher estimate of initial dry mass for *P. pinnatum*, because the final dry mass did not vary among species. Across the full dataset, mean relative growth rate decreased with increasing wood density ($R^2 = 0.43$, $P = 0.008$, $n = 15$).

Cumulative transpiration over the full experiment ranged from 1080 to 3887 kg and was higher on average in *T. grandis* than in *S. macrophylla* or *P. pinnatum* (Figure 2). The amount of water transpired for a given increase in plant dry mass was similar in *S. macrophylla* and *P. pinnatum*, but significantly higher in *T. grandis* ($P < 0.0001$). Thus, *S. macrophylla* and *P. pinnatum* transpired about 2000 kg of water for an 8 kg increase in plant dry mass, whereas *T. grandis* transpired about 3000 kg of water for an equivalent increase in dry mass (Figure 2).

Mean daytime transpiration, calculated from pot weights at dawn and dusk in the week preceding harvest, was significantly correlated with sapling leaf area at harvest (Figure 3). Relationships between daytime transpiration

Table 1. Morphological and physiological characteristics of trees at the conclusion of the experiment. The TE was calculated both with and without leaf litter production. Final plant dry mass does not include leaf litter production. Values are given as mean, with 1 SD in parentheses. For each species, $n = 5$. Values within a row followed by different letters are significantly different at $P < 0.05$.

| | <i>T. grandis</i> | <i>S. macrophylla</i> | <i>P. pinnatum</i> |
|---|-------------------|-----------------------|--------------------|
| Final plant dry mass (kg) | 7.75 (2.66) a | 8.35 (1.76) a | 6.16 (2.51) a |
| Leaf area (m ²) | 11.3 (3.5) a | 10.4 (4.1) a | 7.5 (2.9) a |
| Stem height (m) | 5.99 (1.02) a | 4.77 (0.84) a,b | 4.54 (0.65) b |
| Stem diameter at 1.3 m (cm) | 5.98 (0.74) a | 6.64 (0.59) a | 4.24 (0.48) b |
| Stem wood density (g cm ⁻³) | 0.51 (0.04) a | 0.51 (0.08) a | 0.65 (0.03) b |
| Leaf/plant dry mass ratio (g g ⁻¹) | 0.15 (0.02) a | 0.17 (0.05) a | 0.14 (0.01) a |
| Stem/plant dry mass ratio (g g ⁻¹) | 0.52 (0.03) a | 0.63 (0.04) b | 0.54 (0.03) a |
| Root/plant dry mass ratio (g g ⁻¹) | 0.32 (0.05) a | 0.19 (0.02) b | 0.33 (0.02) a |
| Root/shoot dry mass ratio (g g ⁻¹) | 0.48 (0.11) a | 0.24 (0.03) b | 0.49 (0.04) a |
| Leaf area ratio (m ² kg ⁻¹) | 1.47 (0.12) a | 1.27 (0.57) a | 1.25 (0.24) a |
| Mean relative growth rate (mg g ⁻¹ day ⁻¹) | 11.8 (0.8) a | 12.5 (0.4) a | 9.8 (0.7) b |
| Leaf litter production (kg) | 1.29 (0.53) a | 0.53 (0.06) b | 0.26 (0.06) b |
| TE, including leaf litter (g dry mass kg ⁻¹ H ₂ O) | 2.91 (0.48) a | 4.34 (0.40) b | 4.12 (0.23) b |
| TE, excluding leaf litter (g dry mass kg ⁻¹ H ₂ O) | 2.49 (0.50) a | 4.07 (0.43) b | 3.94 (0.26) b |
| Photosynthetic rate (μmol CO ₂ m ⁻² s ⁻¹) | 17.1 (1.7) a | 16.2 (1.5) a | 20.9 (1.7) b |
| Stomatal conductance to H ₂ O (mol m ⁻² s ⁻¹) | 0.70 (0.07) a | 0.51 (0.05) b | 0.67 (0.06) a |
| Instantaneous transpiration (mmol m ⁻² s ⁻¹) | 5.4 (0.5) a | 4.7 (0.4) a | 5.4 (0.4) a |
| Instantaneous c_i/c_a | 0.85 (0.01) a | 0.82 (0.01) b | 0.81 (0.01) b |
| Stem-wood δ ¹³ C (‰) | -28.1 (0.5) a | -27.1 (0.3) b | -28.2 (0.4) a |

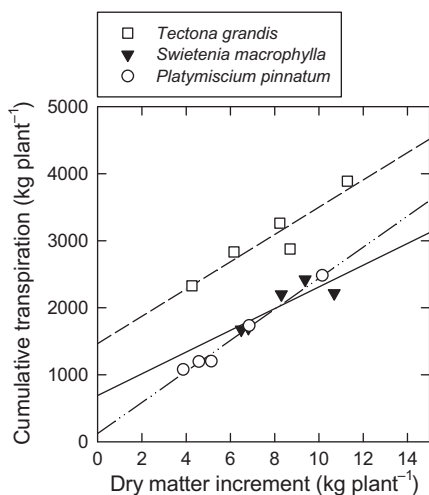


Figure 2. Cumulative transpiration over the course of the experiment plotted against the dry matter increment over the same time period. Dry matter increment refers to the increase in plant dry mass and does not include leaf litter production. Lines are least-squares linear regressions for each species. The dashed line refers to *T. grandis*, the solid line refers to *S. macrophylla* and the dash-dotted line refers to *P. pinnatum*.

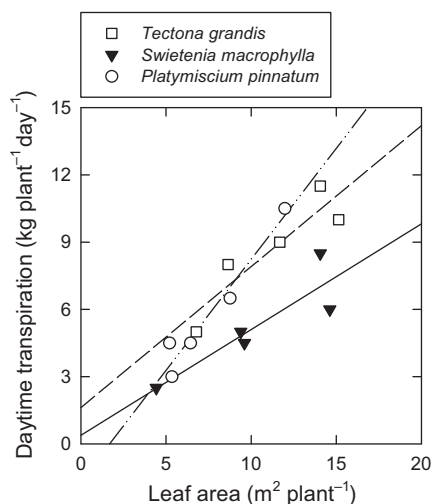


Figure 3. Transpiration over the course of a day plotted against sapling leaf area. Transpiration was determined by weighing pots at dawn and dusk. Leaf area was determined by destructive harvest. Lines are least-squares linear regressions for each species. The dashed line refers to *T. grandis*, the solid line refers to *S. macrophylla* and the dash-dotted line refers to *P. pinnatum*.

and sapling leaf area varied among species ($P = 0.007$). For a given sapling leaf area, *S. macrophylla* transpired significantly less than *T. grandis* or *P. pinnatum* (Figure 3). When daytime transpiration was normalized for leaf area, it was significantly correlated with instantaneous measurements of stomatal conductance (Figure 4). Thus, cuvette-based measurements of stomatal conductance explained 75% of variation in gravitational measurements of sapling

transpiration when transpiration was expressed on a leaf area basis ($R^2 = 0.75$, $P < 0.0001$, $n = 15$). *Swietenia macrophylla* had a significantly lower stomatal conductance than *T. grandis* or *P. pinnatum* (Table 1).

The TE of dry matter production was significantly lower in *T. grandis* than in *S. macrophylla* or *P. pinnatum* (Table 1). Values of TE were calculated to include leaf litter production. Excluding leaf litter from the calculation led to

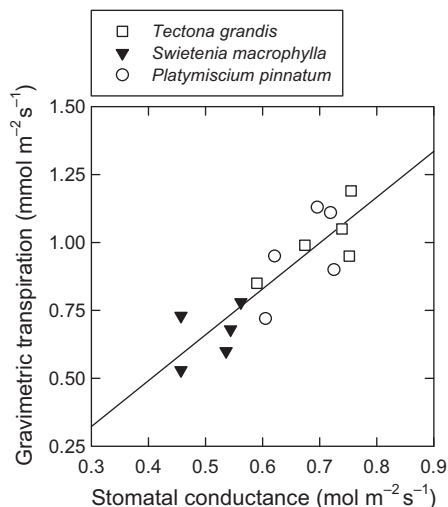


Figure 4. Transpiration determined gravimetrically for whole plants plotted against instantaneous measurements of stomatal conductance. Instantaneous measurements were performed on eight leaves per sapling. Gravimetric measurements integrated over a full day from dawn until dusk. The solid line is a least-squares linear regression through all data points.

larger differences in TE between *T. grandis* and the other two species (Table 1). The TE was significantly correlated with instantaneous measurements of c_i/c_a , both for TE including leaf litter (Figure 5A) and for TE excluding leaf litter (Figure 5C). Instantaneous c_i/c_a explained 66% of variation in TE across the full dataset ($R^2 = 0.66$, $P = 0.0003$, $n = 15$), and a similar correlation was observed when leaf litter production was not included in the calculation of TE ($R^2 = 0.67$, $P = 0.0002$, $n = 15$). The c_i/c_a was significantly higher in *T. grandis* than in *S. macrophylla* or *P. pinnatum* (Table 1).

Measurements of stem-wood $\delta^{13}\text{C}$ explained 28% of variation in TE ($R^2 = 0.28$, $P = 0.04$, $n = 15$), or 23% when leaf litter production was excluded from the calculation of TE ($R^2 = 0.23$, $P = 0.07$, $n = 15$). Scatter in the correlation between TE and $\delta^{13}\text{C}$ was mostly caused by *P. pinnatum* (Figure 5B and D). If *P. pinnatum* were excluded from the dataset, measurements of stem-wood $\delta^{13}\text{C}$ explained 78% of variation in TE for the remaining two species, both with leaf litter included and excluded from the calculation of TE ($R^2 = 0.78$, $P = 0.0007$, $n = 10$). Stem-wood $\delta^{13}\text{C}$ was lower in *S. macrophylla* than in *T. grandis* or

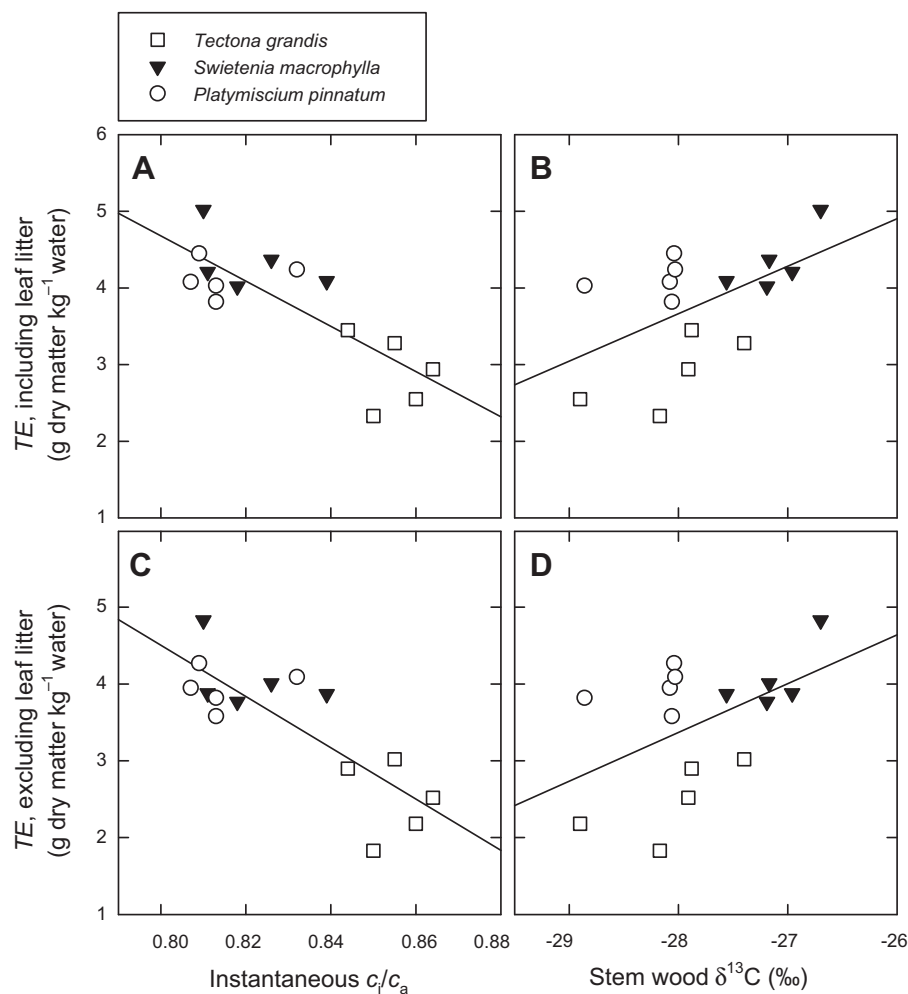


Figure 5. The TE determined over the full 16-month experiment plotted against instantaneous measurements of c_i/c_a (A, C) and stem-wood $\delta^{13}\text{C}$ (B, D). The top two panels show TE with leaf litter production included, and the bottom two panels show TE excluding leaf litter production. Solid lines are least-squares linear regressions.

P. pinnatum and was similar between the latter two species (Table 1). Across the full dataset, stem-wood $\delta^{13}\text{C}$ and instantaneous c_i/c_a were not significantly correlated with each other ($R^2 = 0.07$, $P = 0.35$, $n = 15$). Within individual species, stem-wood $\delta^{13}\text{C}$ tended to be a better predictor of TE than instantaneous c_i/c_a (Figure 5). Thus, an analysis of covariance among species with stem-wood $\delta^{13}\text{C}$ as covariate explained 86% of variation in TE, whereas a similar analysis with instantaneous c_i/c_a as covariate explained 79% of variation in TE.

Discussion

We compared the TE of saplings over a full annual cycle for three tropical tree species and tested the relationships between TE and instantaneous c_i/c_a and stem-wood $\delta^{13}\text{C}$. We observed that TE of *T. grandis* saplings was significantly lower than that of *S. macrophylla* or *P. pinnatum* saplings, whereas the latter two species did not differ. Across the three species, instantaneous measurements of c_i/c_a correlated well with TE (Figure 5A); the c_i/c_a explained 66% of variation observed in TE. This was remarkable, given that measurements of TE integrated over 16 months, whereas measurements of c_i/c_a integrated over minutes. On the other hand, stem-wood $\delta^{13}\text{C}$ explained only 28% of variation in TE across the full dataset. This was surprising because stem-wood $\delta^{13}\text{C}$ integrated over a similar time period as TE. However, the weak correlation between stem-wood $\delta^{13}\text{C}$ and TE appeared to have been caused mainly by the results of one species, *P. pinnatum* (Figure 5B), and within individual species stem-wood $\delta^{13}\text{C}$ was a better predictor of variation in TE than instantaneous c_i/c_a .

We observed that TE of *S. macrophylla* was about 50% higher than that of *T. grandis* (Table 1). This difference is similar in magnitude to that observed previously between the two species (Winter et al. 2005, Cernusak et al. 2007a, 2008). The results given in Table 1 show that this difference persists in saplings, and that it is not affected by integration over both wet and dry seasons. Both *S. macrophylla* (mahogany) and *T. grandis* (teak) are commercial timber species, which are widely planted throughout the tropics. Physiological data about these species may, therefore, be useful for modeling growth and water use of forest plantations. Data presented in Figure 5A suggest that variation in TE between *S. macrophylla* and *T. grandis* was primarily caused by the variation in c_i/c_a , rather than by other processes that can potentially affect TE, such as respiratory carbon use and nighttime water loss. For these two species, the variation in c_i/c_a appeared to be associated with the variation in stomatal conductance, which was consistent with a previous analysis (Cernusak et al. 2008) and with the observations in some other tropical tree species (Guehl et al. 2004).

The leguminous tree species *P. pinnatum* was previously observed to have a higher TE than *S. macrophylla* or

T. grandis when grown in 381 pots (Cernusak et al. 2007a, 2008). However, in a recent experiment, we observed that the advantage in TE of *P. pinnatum* over the other two species declined as the soil nutrient availability increased (Cernusak et al. 2009b). *Platymiscium pinnatum* forms nodules on its roots and is presumably capable of symbiotic fixation of atmospheric nitrogen. Thus, it may be able to maintain a higher TE when soil nitrogen is unavailable, because it can supplement its nitrogen supply through atmospheric nitrogen fixation, thereby achieving higher leaf nitrogen contents, higher photosynthetic capacity and lower c_i/c_a . In this experiment, a slow-release fertilizer was initially added to the pots. Additionally, a lower proportion of rice husks was added to the forest topsoil compared to that in previous experiments (Cernusak et al. 2007a, 2008). While improving the soil structure, the rice husks also have a high C/N ratio and therefore reduce nitrogen availability (Cernusak et al. 2007b). Thus, the combination of fertilizer addition, a lower proportion of rice husks and the large pot size in this experiment may have resulted in increased soil nitrogen availability compared to previous experiments, allowing *S. macrophylla* to achieve a similar TE to *P. pinnatum*. Although *P. pinnatum* had a similar stomatal conductance to *T. grandis*, it had a higher photosynthetic rate (Table 1), suggesting that photosynthetic capacity may have played a role in controlling the difference in TE between these latter two species.

We observed an excellent correspondence between instantaneous measurements of c_i/c_a and annual TE across the full dataset (Figure 5A). This result suggests that variation in instantaneous c_i/c_a , measured under standardized conditions, was representative of variation in c_i/c_a over the full course of the experiment, and that variation in water-use efficiency at the whole-plant scale reflected a variation in water-use efficiency at the leaf scale. It has previously been suggested that variation in water loss not associated with photosynthesis (Hobbie and Colpaert 2004) or respiratory carbon use (Guehl et al. 1994, 1995) can play a large role in controlling variation in TE. The results of this experiment and previous analyses (Cernusak et al. 2007b, 2008) suggest that in tropical trees, c_i/c_a is the primary determinant of TE.

Compared to instantaneous c_i/c_a , stem-wood $\delta^{13}\text{C}$ explained less variation in TE across species. *Platymiscium pinnatum* had a significantly higher TE than *T. grandis*, but both had a very similar stem-wood $\delta^{13}\text{C}$ (Table 1). Thus, based on an analysis of stem-wood $\delta^{13}\text{C}$, one would fail to detect the 40% higher TE in *P. pinnatum* than in *T. grandis*. Additionally, stem-wood $\delta^{13}\text{C}$ was not correlated with instantaneous c_i/c_a in this experiment. However, the range of c_i/c_a and $\delta^{13}\text{C}$ was small. Combining the data from this experiment with the data for the same species from the two previous experiments (Cernusak et al. 2008, 2009b) provides a larger range of values for comparison. For the combined dataset, instantaneous c_i/c_a explained 70% of variation in stem-wood $\delta^{13}\text{C}$

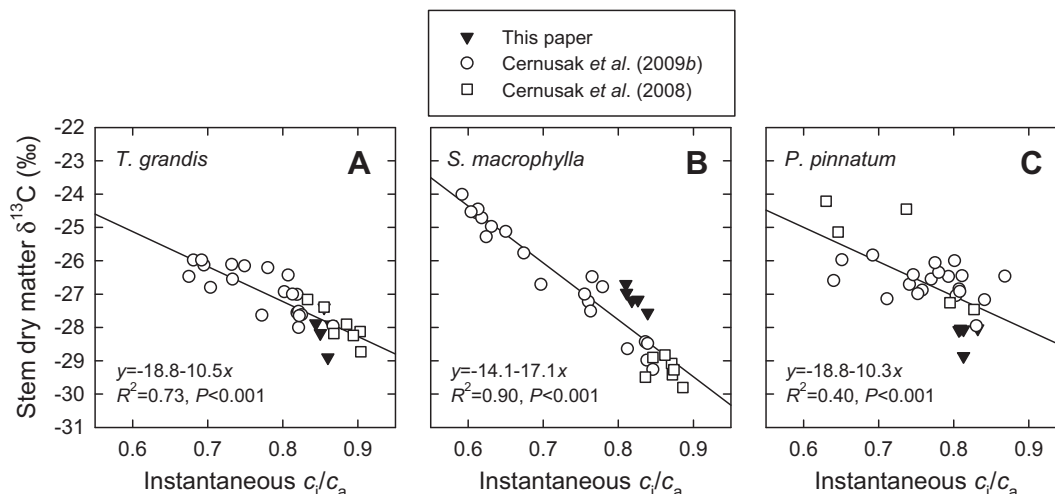


Figure 6. Relationships between stem dry matter $\delta^{13}\text{C}$ and instantaneous measurements of c_i/c_a in (A) *T. grandis*, (B) *S. macrophylla* and (C) *P. pinnatum*. Regression coefficients and statistics are shown in each panel. For *T. grandis* and *S. macrophylla*, $n = 32$; for *P. pinnatum*, $n = 30$. The figures contain data from two previous publications (Cernusak et al. 2008, 2009b), in addition to the data given in this study.

($R^2 = 0.70$, $P < 0.0001$, $n = 94$); the c_i/c_a ranged from ~ 0.6 to 0.9 , and the stem-wood $\delta^{13}\text{C}$ ranged from about -24‰ to -30‰ . This combined analysis clearly supports the use of stem-wood $\delta^{13}\text{C}$ as an indicator of variation in c_i/c_a . Correlations within species are shown in Figure 6A–C. The slope of the relationship appears to be higher for *S. macrophylla* than for the other two species, and the relationship for *P. pinnatum* contains rather more scatter than that for *S. macrophylla* or *T. grandis* (Figure 6). Thus, there appears to be an element of species specificity in the relationships between stem-wood $\delta^{13}\text{C}$ and instantaneous c_i/c_a .

The stem disks that were taken at 0.8-m height for the analysis of $\delta^{13}\text{C}$ were produced entirely during the experimental period, because the seedlings were initially less than 0.8-m tall. Additionally, variation among species in the $\delta^{13}\text{C}$ of atmospheric CO_2 can be ruled out, because the saplings were randomly distributed in an open area, well away from the forest edge. Several possibilities exist that could cause stem-wood $\delta^{13}\text{C}$ to diverge from the $\delta^{13}\text{C}$ of the initial products of photosynthesis. These have been recently reviewed (Hobbie and Werner 2004, Badeck et al. 2005, Bowling et al. 2008, Cernusak et al. 2009a) and include respiratory processes (Duranceau et al. 1999, Cernusak and Marshall 2001, Ocheltree and Marshall 2004, Klumpp et al. 2005, Bathellier et al. 2008), diel carbohydrate dynamics (Tcherkez et al. 2004, Gessler et al. 2008) and refixation of respired CO_2 by photosynthetic bark (Cernusak et al. 2001, 2006). Further investigation into what causes variation among species in the relationship between c_i/c_a and stem-wood $\delta^{13}\text{C}$ would be very useful for water-use efficiency research.

The correlation shown in Figure 4 suggests that variation in whole-sapling transpiration rates, when expressed on a

leaf area basis, was closely associated with variation in stomatal conductance. In this experiment, the saplings were spaced apart by 3–4 m, such that their crowns were isolated from one another, and they were grown in the open, away from the forest edge. For tropical trees growing in closed-canopy stands, significant uncoupling between stomatal conductance and whole-tree transpiration has been observed (Meinzer et al. 1993, 1995, 1997), suggesting that in such a situation, boundary layer resistance to water vapor diffusion is a significant component of the total resistance. The close correspondence between stomatal conductance and whole-sapling transpiration in this experiment suggests that canopy boundary layer resistance was likely smaller than stomatal resistance under our experimental conditions.

Biomass allocation patterns varied among the three species (Table 1). *Swietenia macrophylla* had a larger stem mass ratio, defined as stem dry mass divided by total plant dry mass, than the other two species. This larger allocation to stem biomass could be of benefit for forestry applications if it persists through stand development, because it would mean that the harvestable fraction of tree biomass would be higher than in the other two species. Root mass ratio and root/shoot ratio were also lower in *S. macrophylla* than in the other two species (Table 1). It would be of interest to know if these variations in biomass allocation persist in larger trees and under varying soil conditions, because they could have implications for the estimation of belowground biomass in tropical forestry plantations.

In conclusion, we observed a significant variation in TE among the three tropical tree species grown in large pots over a full annual cycle. This result builds on previous analyses demonstrating that *S. macrophylla* has a higher TE than *T. grandis*. Whereas *P. pinnatum* had

displayed a higher TE than *S. macrophylla* or *T. grandis* in previous experiments, it showed a similar TE to *S. macrophylla* when grown in large pots in lightly fertilized soil. This difference in the relative performance of *P. pinnatum* may be related to variation in availability of soil nitrogen between this experiment and the previous experiments. Variation in TE across the full dataset corresponded closely with variation in instantaneous measurements of c_i/c_a , suggesting that variation in water-use efficiency at the whole-plant level reflected that at the leaf level. Variation in TE between *P. pinnatum* and *T. grandis* was not reflected in stem-wood $\delta^{13}\text{C}$, suggesting that cross-species comparisons of TE based on the measurements of stem-wood $\delta^{13}\text{C}$ may lack sensitivity in some cases. However, analysis of a combined dataset, including the previously published data for the same three species, clearly supported the utility of stem-wood $\delta^{13}\text{C}$ as an indicator of variation in c_i/c_a (Figure 6).

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References

- Bacon, M.A. 2004. Water use efficiency in plant biology. *In* Water Use Efficiency in Plant Biology. Ed. M.A. Bacon. Blackwell Publishing, Oxford, pp 1–26.
- Badeck, F.-W., G. Tcherkez, S. Nogues, C. Piel and J. Ghashghaie. 2005. Post-photosynthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon. *Rapid Commun. Mass Spectrom.* 19:1381–1391.
- Bathellier, C., F.-W. Badeck, P. Couzi, S. Harscoët, C. Mauve and J. Ghashghaie. 2008. Divergence in $\delta^{13}\text{C}$ of dark respired CO_2 and bulk organic matter occurs during the transition between heterotrophy and autotrophy in *Phaseolus vulgaris* plants. *New Phytol.* 177:406–418.
- Bowling, D.R., D.E. Pataki and J.T. Randerson. 2008. Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. *New Phytol.* 178:24–40.
- Briggs, L.J. and H.L. Shantz. 1914. Relative water requirement of plants. *J. Agric. Res.* 3:1–64.
- Cernusak, L.A. and J.D. Marshall. 2001. Responses of foliar $\delta^{13}\text{C}$, gas exchange, and leaf morphology to reduced hydraulic conductivity in *Pinus monticola* branches. *Tree Physiol.* 21:1215–1222.
- Cernusak, L.A., J.D. Marshall, J.P. Comstock and N.J. Balser. 2001. Carbon isotope discrimination in photosynthetic bark. *Oecologia* 128:24–35.
- Cernusak, L.A., L. Hutley, J. Beringer and N.J. Tapper. 2006. Stem and leaf gas exchange and their responses to fire in a north Australian tropical savanna. *Plant Cell Environ.* 29:632–646.
- Cernusak, L.A., J. Aranda, J.D. Marshall and K. Winter. 2007a. Large variation in whole-plant water-use efficiency among tropical tree species. *New Phytol.* 173:294–305.
- Cernusak, L.A., K. Winter, J. Aranda, B.L. Turner and J.D. Marshall. 2007b. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. *J. Exp. Bot.* 58:3549–3566.
- Cernusak, L.A., K. Winter, J. Aranda and B.L. Turner. 2008. Conifers, angiosperm trees, and lianas: growth, whole-plant water and nitrogen use efficiency, and stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) of seedlings grown in a tropical environment. *Plant Physiol.* 148:642–659.
- Cernusak, L.A., G. Tcherkez, C. Keitel et al. 2009a. Why are non-photosynthetic tissues generally ^{13}C enriched compared to leaves in C_3 plants? Review and synthesis of current hypotheses. *Funct. Plant Biol.* 36:199–213.
- Cernusak, L.A., K. Winter and B.L. Turner. 2009b. Physiological and isotopic ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) responses of three tropical tree species to water and nutrient availability. *Plant Cell Environ.* (in press), doi: 10.1111/j.1365-3040.2009.02010.x.
- Duquesnay, A., N. Bréda, M. Stievenard and J.L. Dupouey. 1998. Changes of tree-ring $\delta^{13}\text{C}$ and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant Cell Environ.* 21:565–572.
- Duranceau, M., J. Ghashghaie, F. Badeck, E. Deleens and G. Cornic. 1999. $\delta^{13}\text{C}$ of CO_2 respired in the dark in relation to $\delta^{13}\text{C}$ of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought. *Plant Cell Environ.* 22:515–523.
- Eamus, D. 1991. The interaction of rising CO_2 and temperatures with water use efficiency. *Plant Cell Environ.* 14:843–852.
- Farquhar, G.D. 1997. Carbon dioxide and vegetation. *Science* 278:1411.
- Farquhar, G.D. and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency in wheat genotypes. *Aust. J. Plant Physiol.* 11:539–552.
- Farquhar, G.D., M.H. O’Leary and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* 9:121–137.
- Feng, X. 1998. Long-term c_i/c_a response of trees in western North America to atmospheric CO_2 concentration derived from carbon isotope chronologies. *Oecologia* 117:19–25.
- Fischer, R.A. and N.C. Turner. 1978. Plant productivity in the arid and semiarid zones. *Annu. Rev. Plant Physiol.* 29:277–317.
- Gedney, N., P.M. Cox, R.A. Betts, O. Boucher, C. Huntingford and P.A. Stott. 2006. Detection of a direct carbon dioxide effect in continental river runoff records. *Nature* 439:835–838.
- Gessler, A., G. Tcherkez, A.D. Peuke, J. Ghashghaie and G.D. Farquhar. 2008. Experimental evidence for diel variations of the carbon isotope composition in leaf, stem and phloem sap organic matter in *Ricinus communis*. *Plant Cell Environ.* 31:941–953.
- Guehl, J.M., C. Picon, G. Aussenac and P. Gross. 1994. Interactive effects of elevated CO_2 and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. *Tree Physiol.* 14:707–724.
- Guehl, J.M., C. Fort and A. Ferhi. 1995. Differential response of leaf conductance, carbon isotope discrimination and water use efficiency to nitrogen deficiency in maritime pine and pedunculate oak plants. *New Phytol.* 131:149–157.

- Guehl, J.M., D. Bonal, A. Ferhi, T.S. Barigah, G.D. Farquhar and A. Granier. 2004. Community-level diversity of carbon-water relations in rainforest trees. *In* Ecology and Management of a Neotropical Rainforest. Eds. S. Gourlet-Fleury, J.M. Guehl and O. Laroussinie. Elsevier, San Diego, CA, pp 75–94.
- Hobbie, E.A. and J.V. Colpaert. 2004. Nitrogen availability and mycorrhizal colonization influence water use efficiency and carbon isotope patterns in *Pinus sylvestris*. *New Phytol.* 164:515–525.
- Hobbie, E.A. and R.A. Werner. 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C₃ and C₄ plants: a review and synthesis. *New Phytol.* 161:371–385.
- Klumpp, K., R. Schäufele, M. Lötscher, F.A. Lattanzi, W. Feneis and H. Schnyder. 2005. C-isotope composition of CO₂ respired by shoots and roots: fractionation during dark respiration? *Plant Cell Environ.* 28:241–250.
- Lawes, J.B. 1850. Experimental investigation into the amount of water given off by plants during their growth; especially in relation to the fixation and source of their various constituents. *J. Hort. Soc. Lond.* 5:38–63.
- Long, S.P., P.K. Farage and R.L. Garcia. 1996. Measurement of leaf and canopy photosynthetic CO₂ exchange in the field. *J. Exp. Bot.* 47:1629–1642.
- Marshall, J.D. and R.A. Monserud. 1996. Homeostatic gas-exchange parameters inferred from ¹³C/¹²C in tree rings of conifers. *Oecologia* 105:13–21.
- Mcculloh, K.A., K. Winter, F.C. Meinzer, M. Garcia, J. Aranda and B. Lachenbruch. 2007. A comparison of daily water use estimates derived from constant-heat sap-flow probe values and gravimetric measurements in pot-grown saplings. *Tree Physiol.* 27:1355–1360.
- Meinzer, F.C., G. Goldstein, N.M. Holbrook, P. Jackson and J. Cavelier. 1993. Stomatal and environmental control of transpiration in a lowland tropical forest tree. *Plant Cell Environ.* 16:429–436.
- Meinzer, F.C., G. Goldstein, P. Jackson, N.M. Holbrook, M.V. Gutierrez and J. Cavelier. 1995. Environmental and physiological regulation of transpiration in tropical forest gap species: the influence of boundary layer and hydraulic properties. *Oecologia* 101:514–522.
- Meinzer, F.C., J.L. Andrade, G. Goldstein, N.M. Holbrook, J. Cavelier and P. Jackson. 1997. Control of transpiration from the upper canopy of a tropical forest: the role of stomatal, boundary layer and hydraulic architecture components. *Plant Cell Environ.* 20:1242–1252.
- Morison, J.I.L. 1985. Sensitivity of stomata and water use efficiency to high CO₂. *Plant Cell Environ.* 8:467–474.
- Ocheltree, T.W. and J.D. Marshall. 2004. Apparent respiratory discrimination is correlated with growth rate in the shoot apex of sunflower (*Helianthus annuus*). *J. Exp. Bot.* 55:2599–2605.
- Osório, J., M.M. Chaves and J.S. Pereira. 1998. Effects of water deficits on ¹³C discrimination and transpiration efficiency of *Eucalyptus globulus* clones. *Aust. J. Plant Physiol.* 25: 645–653.
- Shantz, H.L. and L.N. Piemeisel. 1927. The water requirement of plants at Akron, Colo. *J. Agric. Res.* 34:1093–1190.
- Sun, Z.J., N.J. Livingston, R.D. Guy and G.J. Ethier. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant Cell Environ.* 19:887–894.
- Tcherkez, G., G. Farquhar, F. Badeck and J. Ghashghaie. 2004. Theoretical considerations about carbon isotope distribution in glucose of C-3 plants. *Funct. Plant Biol.* 31:857–877.
- Winter, K., J. Aranda, M. Garcia, A. Virgo and S.R. Paton. 2001. Effect of elevated CO₂ and soil fertilization on whole-plant growth and water use in seedlings of a tropical pioneer tree, *Ficus insipida* Willd. *Flora* 196:458–464.
- Winter, K., J. Aranda and J.A.M. Holtum. 2005. Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Funct. Plant Biol.* 32:381–388.
- Woodward, J. 1699. Some thoughts and experiments concerning vegetation. *Phil. Trans. R. Soc. Lond.* 21:193–227.
- Zhang, J.W. and J.D. Marshall. 1994. Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Can. J. For. Res.* 24:92–99.