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A comparison of heat pulse and deuterium tracing techniques for estimating sap flow in *Eucalyptus grandis* trees

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Summary Sap flow rates were measured simultaneously by the heat pulse and deuterium tracing techniques in nine *Eucalyptus grandis* W. Hill *ex* Maiden. trees at two sites (1) to compare results from the two techniques and (2) to assess the impact of the assumptions underlying the deuterium tracing method on the calculation of sap flow for a range of tree sizes. The trees ranged in height from 4 to 14 m with leaf areas of 5 to 35 m^2. In all trees, sap flow estimated by the deuterium tracing technique was higher than sap flow estimated by the heat pulse method, with differences of 11 to 43% in eight of the trees and 113% in one tree. The largest difference was attributed to errors in the heat pulse method, as indicated by aberrant relationships between sap flow measured by the heat pulse method and tree size characteristics (i.e., diameter, sap wood area, leaf area) for that tree compared with the other experimental trees. Drilling holes in the trees to allow injection of deuterium had no significant effect on sap flow, even when 32 holes were drilled. Sap flow measured by the heat pulse method was only lower after drilling than before drilling in three trees, and the difference only persisted for about 1 h. Deuterium concentrations of water collected from the tree canopies had not returned to background values 17 days after injection. Twenty-one days after injection, sapwood and heartwood samples taken from trunks near the injection sites contained considerable concentrations of deuterium, indicating that some of the deuterium injected into the trees was still present. An experiment performed on two trees showed that deuterium was stored in the heartwood and sapwood throughout the trees, and its distribution within the trees four days after injection was similar whether it was injected into only the sapwood (where it should mix with sap and be transported from the tree most readily) or into both the sapwood and heartwood, indicating that there was considerable movement of deuterium between the heartwood and sapwood. Deuterium storage was accounted for by an approximate means in the sap flow calculations, and may have resulted in an error of about 10% in sap flow estimated by this method. We conclude that the heat pulse and deuterium tracing techniques can be used simultaneously to increase the number of sap flow measurements obtained from a forest, thereby increasing the precision of forest water use estimates. Their combination would be most effective in stands with a wide range of tree sizes and sap flow rates, where the relative differences in sap flux estimates between the methods is small compared with differences in sap flow between trees.

Keywords: heartwood, leaf area, sapwood, transpiration, water use, xylem.

Introduction

A variety of techniques have been developed to measure transpiration rates from forests, including micrometeorological techniques (e.g., Denmead 1984, Rose and Sharma 1984), sap flow methods (e.g., Hatton and Vertessy 1990, Dunn and Connor 1993, Thorburn et al. 1993*a*), and chemical tracers (e.g., Calder et al. 1986, Calder 1991, 1992). However, there are major drawbacks associated with many of these techniques. For example, there are limitations of fetch and canopy uniformity associated with micrometeorological methods. A limitation associated with sap flow methods is that they require scaling up from individual trees in order to estimate stand water use (Calder 1992, Hatton et al. 1995). The errors associated with scaling from trees to stands can be in the order of 25% (e.g., Thorburn et al. 1993*a*). However, the precision of scaling estimates increases with the number of individual trees sampled in a stand (Hatton et al. 1995). To increase sample sizes it may be advantageous to employ the heat pulse and deuterium tracing methods simultaneously.

Although both techniques employ tracers to determine sap flow, they have important differences. In particular, they operate on different scales; heat pulse measurements are generally made over lengths of 15 mm and are integrated over several minutes, whereas the deuterium tracing method operates at a scale of meters and is integrated over several days. Thus, the sap flow estimates given by the two methods may differ. Both techniques also rely on several assumptions. Many of the assumptions underlying the heat pulse method have been examined in detail (e.g., the impacts of wounding during probe implantation; Swanson and Whitfield 1981, Olbrich 1991). The assumptions behind the deuterium tracing method have been less widely examined. For example, complete recovery of deuterium (i.e., the applied chemical), which is required in the method, may be difficult when working with large trees (Dye et al. 1992). The extent of deuterium storage in trees has not been examined. Additionally, the deuterium tracing method requires that many holes be drilled in the trees for injection of the tracer. These holes will cause disruption to xylem flow (Doley and Grieve 1966), the practical impact of which is unclear.

Before measurements from the two sap flow techniques can be combined to estimate forest transpiration, more information is needed on how results from the methods compare and how some of the assumptions behind the deuterium tracing affect sap flow over a range of tree sizes. To obtain this information, we applied the heat pulse and deuterium tracing methods to estimate sap flux simultaneously in *Eucalyptus grandis* W. Hill *ex* Maiden. trees at two sites.

Materials and methods

Site details

Comparisons of the heat pulse and deuterium tracing techniques were carried out at two sites in southeastern Queensland, Australia, during spring 1994. The first experiment was conducted at Warrill View (152°36′ E, 27°50′ S), 80 km west of Brisbane, on two 3.5-year-old *E. grandis* trees (Trees W1 and W2, Table 1), located on a slope adjacent to a saline discharge zone. Trees at this site were planted in rows 10 m apart, with approximately 3 m between trees in each row. Average annual rainfall at Warrill View is around 870 mm and Class A pan evaporation is about 1500 mm (Dunn et al. 1994). During the experimental period there was no rainfall.

The second experiment was undertaken in a State Forest near Toolara (152°50′ E, 26° S), approximately 200 km north of Brisbane. Ten, 5-year-old *E. grandis* trees (Trees T1 to T10, Table 1) were studied within a planted stand of approximately 500, of which seven (Trees T1 to T7) had both heat pulse and deuterium techniques applied, and three (Trees T8 to T10) only had the heat pulse technique applied. The experimental trees were located in two rows in the southwestern portion of the plot and were chosen to represent the range of tree sizes within the stand. The site occupies the floodplain of a permanent creek. Average annual rainfall at the site is about 1340 mm and Class A pan evaporation is about 1550 mm. During the experimental period rain fell on five days, totalling 30 mm. Of this total, 20 mm fell on one day. Weather data were recorded hourly at automatic weather stations located within 2 km of each site.

Diameter at breast height (DBH), sapwood area and leaf area were measured on all of the experimental trees. Leaf areas of Trees W1 and W2 were measured nondestructively (Andrew et al. 1979). Destructive estimates of leaf area for the 10 trees studied at Toolara were made 10 days after sampling ceased. Volumetric wood and water contents were determined (Anon. 1992) in samples taken from the experimental trees at Warrill View and Toolara at the end of the experiments.

Table 1. Summary of experimental tree characteristics at Warrill View (W) and Toolara (T), southeastern Queensland. Abbreviation: DBH = stem diameter at breast height (1.3 m).

Tree	Height	DBH	Sapwood area	Leaf area	Volume fraction	
	(m)	(mm)	(mm^2)	(m^2)	Wood	Water
W1	4.5	66	2.83	5.6	0.40	0.56
W2	4.3	66	2.83	6.7	0.40	0.57
T1	12.8	126	8.30	27.8	0.42	0.53
T2	12.9	128	8.85	34.6	0.37	0.54
T3	12.2	118	8.37	23.2	0.47	0.51
T4	9.0	66	2.63	5.1	0.45	0.53
T5	12.7	112	8.04	17.9	0.42	0.54
T6	13.5	134	10.56	34.6	0.41	0.51
T7	11.9	106	6.29	18.2	0.39	0.55
T8	11.8	132	10.94	23.9	0.41	0.52
T9	12.0	118	8.70	31.4	0.39	0.55
T10	10.5	110	7.47	20.4	0.42	0.50

Heat pulse method

The probe sets used to estimate sap flow by the heat pulse method (Huber and Schmidt 1937) consisted of two sensor probes and a heater probe (SF100 units, Greenspan Technology, Warwick, Australia). Each sensor probe consisted of two thermistors, situated 5 mm apart. The upstream and downstream sensor probes were 5 mm below and 10 mm above the heater probe, respectively. Two probe sets were used per tree giving measurements at four radial positions in the sapwood.

Because the small holes that are drilled in the stem of the tree for probe emplacement disrupt the xylem vessels in the region around the probes, changing the thermal properties of the wood and interrupting sap flow (Swanson and Whitfield 1981), the extent of the flow disruption (known as the wound width) needs to be accounted for. Wound widths were measured in sections taken from around each of the four thermistor probe holes per tree by making a horizontal cut through the length of each probe hole. The sectioned piece of timber was then sanded with fine-grade glass paper. Dark staining of wood on either side of the probe hole could be clearly seen with the aid of a dissecting microscope. The diameter of the stained wood corresponds to the extent of vessels blocked as a result of drilling (the wound width) (J. Marshall, CSIRO, Division of Water Resources, Perth, Australia, personal communication 1995). Four wound width measurements were made on each sensor probe hole, resulting in 16 measurements per tree. A single mean value was then determined for each tree (Table 2).

Stems of two trees at the Warrill View site were implanted with probe sets located on opposite sides of each tree, 0.3 m above ground. Holes were drilled with a 1.98 mm bit to accommodate the heat pulse probes. A guide rod and drill guide were used to ensure that the holes drilled to accommodate the three probes of the heat pulse unit were correctly spaced and parallel. If probe separation deviated noticeably new holes were drilled. The data loggers were programed to provide a 30 °C heat pulse (1.3 A) for 1.8 s every 20 min.

Because initial results showed that sap flow occurred over the depth of the whole stem, indicating no heartwood develop-

Table 2. Wound widths of 3.5-year-old *E. grandis* trees at Warrill View (W) and 5-year-old *E. grandis* trees at Toolara (T).

Tree	Mean wound width (mm)	Standard deviation	Coefficient of variation (%)
W1	3.0	0.12	4.0
W2	3.1	0.10	3.3
T1	3.5	0.47	13.6
T2	3.1	0.25	8.2
T3	2.8	0.18	6.5
T4	2.5	0.05	2.0
T5	2.5	0.08	3.2
T6	2.7	0.18	7.0
T7	3.0	0.12	4.0
T8	3.4	0.29	8.5
T9	2.6	0.05	1.9
T10	3.0	0.15	5.1
Mean	2.9	0.17	5.6

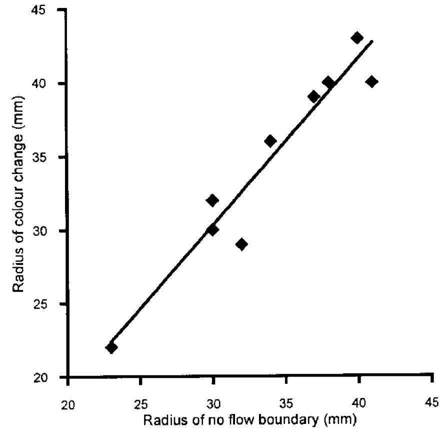

Figure 1. Radius of no-flow boundary determined by the heat pulse method versus mean radius of sapwood conducting area for each tree at Toolara. The $r^2 = 0.93$ ($y = 1.13$ ($\pm$ 0.08)$x - 3.62$ ($\pm$ 2.96)).

ment, probes were implanted with the thermistors spaced equally throughout the sapwood, and the probe sets fixed in place on the guide rods with electrical tape. The entire portion of the trunk encompassing the heat pulse units was then wrapped in reflective foil insulation to act as a solar radiation shield and provide protection from the weather.

Heat pulse measurements were made on 10 trees at the Toolara site following the same method used at Warrill View, with two probe sets installed in opposite sides of each tree at a height of 1.3 m above ground (breast height). Unlike the trees at Warrill View, the trees at Toolara had heartwood. The boundary between the sapwood (conducting wood) and heartwood (non-conducting wood) was identified by progressively moving the probes deeper into the tree and recording the sap flow at each depth. The probes were fixed so that the thermistors were spaced evenly throughout the conducting wood of each tree. Because the conducting wood area of each tree is required to calculate tree sap flux, the trees were cut down after the experiment and the radius of the observed sapwood–heartwood color differentiation measured at four points around the tree, with a mean value determined for each tree. Color differentiation was found to be a good indicator of sapwood–heartwood boundary in the experimental trees (Figure 1).

Deuterium tracing

We used the deuterium tracing technique described by Calder et al. (1986). Deuterium oxide (D_2O) (99.8% minimum purity) was injected into two *E. grandis* trees at Warrill View and seven of the ten trees used for the heat pulse experiment at Toolara. Before injection of D_2O, clear plastic bags were placed around leaves at three canopy levels to condense and collect transpired water vapor. For the Warrill View trees, two bags per canopy level were used, and three bags per canopy level were used for the Toolara trees (a truck-mounted “cherry picker” was used to access the canopies of the Toolara trees). Each bag contained about 15 leaves and was sealed with insulation tape. After one day the bags were removed and condensate samples were collected to determine background D_2O concentrations. New bags were placed around the same leaves on each tree.

The D_2O was injected into 2 mm diameter holes, spaced regularly around the stem of each tree and inclined downward at an angle of 30° to the horizontal, 0.3–0.4 m aboveground. The injection holes were drilled as low as possible in the trees to maximize the distance before the flow stream split to separate branches, ensuring complete mixing of the tracer (Calder et al. 1986). The number of holes per tree varied according to the amount of D_2O injected (Table 3). For each tree, an equal amount of D_2O was injected into each hole, with a syringe, and the hole was then sealed with wood putty. The total amount of D_2O injected into each tree (Table 3) was adjusted—based on initial heat pulse velocities—to yield peak D/H ratios (deuterium to hydrogen isotopic ratios) between 2.3 and 3.1×10^{-4}. This range was a compromise between adding a significant mass of tracer and ensuring that the analytical precision of the samples was maintained.

At Warrill View, condensate samples were collected twice per day for the first 2 days after injection, then daily for the following 10 days. Condensate samples were collected at Toolara daily for the first 9 days after injection and then on Days

Table 3. Injection details of deuterium tracer experiment.

Tree	D_2O injected (ml)	No. of injection holes	Depth of injection holes (mm)	Distance to first live branch (m)
W1	0.35	8	35	1.0
W2	0.35	8	35	1.1
T1	2.5	32	45	4.0
T2	1.3	16	45	3.9
T3	1.2	16	45	4.5
T4	0.9	8	30	4.0
T5	2.4	32	45	3.9
T6	1.3	16	45	3.7
T7	1.2	16	45	4.3

11, 12, 13 and 15 for all trees, with Tree T1 also sampled on Day 10. Based on previous studies (Calder et al. 1986, Calder et al. 1992, Dye et al. 1992), it was estimated that all of the tracer would have passed through the Warrill View trees by Day 12, and through the Toolara trees by Day 14.

Typically, about 4 ml of condensate was collected in each bag per day, a subsample of which was obtained with a syringe. A new syringe was used for each bag to prevent cross-contamination. At Toolara, the samples from each plastic bag on Tree T1 were kept separate, resulting in nine samples per day to provide information on intra-canopy variation in tracer concentration. For Tree T2, an equal amount of condensate from bags of the same canopy level were bulked, resulting in three samples per day. For Trees T3, T4, T5, T6 and T7, and the trees at Warrill View, an equal amount of condensate was drawn from each plastic bag and combined into a single 3 ml sample, resulting in one sample per day for each of these trees.

The deuterium concentration (the deuterium to hydrogen isotopic ratio) of the condensate was then determined and flow rates calculated (Calder 1991):

$$F = \frac{M}{\sum_{i=1}^{T} C_i \Delta t_i} \quad , \tag{1}$$

where M is the mass of injected tracer, C is the concentration of the tracer, i refers to the time increment, Δt_i is the duration of the time increment, and T is the last time increment in which the tracer is present.

Deuterium storage in sapwood and heartwood

An assumption of the deuterium tracing method is that there is no residual deuterium in the tree at the end of the sampling period (Calder et al. 1986). If this is not the case, the denominator on the right hand side in Equation 1 will be underestimated and the flux overestimated. To assess whether any D_2O remained in the trees after the experiments, cores were taken from the tree trunks and analyzed for residual deuterium storage. Three 12-mm diameter cores were taken from each of the injected trees, 100 mm above the injection holes, one week after condensate sampling was concluded (i.e., 21 days after D_2O injection). The sapwood and heartwood portions of the cores were stored separately in airtight containers filled with kerosene. The water was later extracted from the cores by azeotropic distillation (Thorburn et al. 1993*b*) and analyzed for deuterium concentration.

Because a substantial amount of deuterium was found in the heartwood and sapwood, we characterized the extent of deuterium storage in an additional two trees. This experiment was carried out in spring 1995 with two *E. grandis* trees (Tree SW and Tree HW) located in the same portion of the stand at the Toolara site. The trees were of similar size (Tree SW: 13.2 m tall and DBH of 128 mm; Tree HW: 12.3 m tall and DBH of 120 mm), and had similar sap fluxes (Tree SW: 13.8 l day^{-1}; Tree HW: 12.9 l day^{-1}) to the trees used in the previous experiment at Toolara.

Each tree was injected with 1.3 ml of D_2O (99.8% minimum purity), distributed equally into 16 regularly spaced holes, following the same method as described above except for the depth of injection holes. The injection holes in Tree SW were 30 mm deep, and penetrated the sapwood only, whereas the holes were drilled to a depth of 50 mm in Tree HW, ensuring they were well into the heartwood of the tree.

Sapwood samples were taken from both trees (at 3 m above ground) on the day before injection to determine background deuterium concentrations, then 0.5, 1, 4, 8 and 17 days after injection at heights of 0.4, 0.6, 2 and 4 m above ground. Twig samples from the lower canopies of the trees (approximately 9 and 8 m above ground for Trees SW and HW, respectively) were also taken at each sampling time. The D/H ratio of the water in the twigs should be similar to the concentrate collected over a 24-h period, because there is little long-term (greater than 24 h) storage of deuterium in leaves (Forstel 1978, Zundel et al. 1978). The samples from the twigs were therefore used to estimate the D/H ratio of the water being transpired from the trees (i.e., approximating the tracer breakthrough curve). Heartwood samples were taken from both trees at each sample time, except Day 0.5, at heights of 0.4, 0.6 and 2 m above ground (there was no heartwood development at 4 m). Both trees were blown over in a storm 27 days after injection.

Sapwood and heartwood samples were taken with a chisel at various sampling points around the stem. Twig samples were obtained with an extended branch cutter. All samples were immediately placed in kerosene-filled airtight jars until subjected to azeotropic distillation and analysis.

Analysis of deuterium concentration

Analysis of the deuterium concentration in the condensate, and in the heartwood and sapwood samples, was carried out by conventional mass spectrometry. Analyses were performed by reduction of 25 µl of sample to H_2 over uranium at 800 °C. Isotopic concentrations were expressed as the ratio of deuterium to hydrogen isotopes and converted to D/H concentration above background. The background D/H ratio for the Warrill View trees was $154.96 \pm 0.1 \times 10^{-6}$, determined as the mean of the pre-injection condensate samples from the two trees. The background D/H ratio for the Toolara trees was $156.4 \pm 0.19 \times 10^{-6}$, determined as the mean D/H ratio of pre-injection condensate samples from the seven trees. In terms of D/H ratio, the mass spectrometer analyses was precise to $\pm 0.15 \times 10^{-6}$.

Results

Heat pulse determined sap flow

Sap flow rates determined by the heat pulse method generally followed a typical diurnal cycle: increasing shortly after sunrise to reach a peak in the early afternoon, then decreasing in the late afternoon–evening (Figure 2). At Warrill View, mean daily sap flow flux over the experimental period was 4.5 and 4.6 l day^{-1} for Trees W1 and W2, respectively. For the Toolara trees, mean daily sap flow ranged between 2.6 and 21.6 l day^{-1} (Table 4).

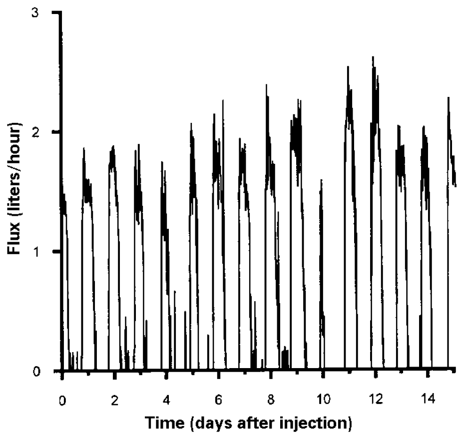

Figure 2. Sap flux estimated by the heat pulse method for Tree T6 at Toolara (t = 0 represents noon on the day of injection).

Total daily sap flow in the Warrill View trees, calculated from heat pulse data, was closely correlated with daily solar radiation (r^2 = 0.88 for Tree W1 and r^2 = 0.93 for Tree W2; n = 12). No solar radiation data were available during the Toolara experimental period; however, daily transpiration decreased during rainy periods, 4 and 10 days after injection (Figure 2).

Nighttime sap flow was recorded for trees at Warrill View and Toolara at various times during the experimental period (Figure 2). However, nighttime sap flow rates were low and were not consistent among the experimental trees, indicating that the measurements were the result of rehydration of xylem above the heat pulse units rather than actual nighttime transpiration. Observed nighttime sap flow flux accounted for approximately 1% of total daily flux.

Effect of drilling holes for deuterium injection

Heat pulse measurements indicated that drilling holes only disrupted sap flow in three trees (Trees T1, T3 and T7) immediately following the drilling. In these trees, sap flow was reduced by 20 to 40% (Figure 3) but it recovered to pre-drilling rates within an hour. There were no discernible changes in sap flow rates in any of the other trees following the drilling of injection holes (although the injection holes in the Warrill View trees were located approximately 120 mm above the heat pulse probes).

Table 4. Comparisons of sap flux estimated by the deuterium tracing and heat pulse methods for 3.5-year-old *E. grandis* trees at Warrill View (W) and 5-year-old *E. grandis* trees at Toolara (T).

Tree	Heat pulse estimated sap flux (l day^{-1})	Deuterium tracing estimated sap flux (l day^{-1})	Difference as % of heat pulse
W1	4.5	5.0	11
W2	4.6	5.5	18
T1	14.5	16.2	12
T2	10.0	21.3	113
T3	10.6	15.0	41
T4	2.6	3.7	43
T5	11.3	15.3	36
T6	21.6	26.1	21
T7	10.3	13.7	32

Deuterium tracing determined sap flow

Daily D/H ratios in the condensate for Trees W1, T1 and T2 (Figure 4) are representative of those from the Warrill View and Toolara trees. For the Warrill View trees, D/H ratios showed a sharp increase on the first sampling day, reaching peaks between one and two days after injection (Figure 4a). By the end of the sampling period at Warrill View (Day 12), D/H ratios were 0.48 and 0.26×10^{-6} above background in Trees W1 and W2, respectively.

Because a basic assumption of the deuterium tracing method is that all of the injected tracer passes through the system during the experiment, we used a linear extrapolation through the last portion of the curve to obtain estimates of when D/H ratios returned to background values (e.g., Figure 5; cf. Dye et al. 1992). Linear extrapolation of the data from Warrill View indicated that end points would have been reached approximately 4 days after the end of sampling for Tree W1 and 2 days after the end of sampling for Tree W2. The denominator on the right hand side in Equation 1 was then calculated using these end-point estimates as values for T, allowing sap flux to be determined (Table 4). Extrapolation to these end points had only a negligible effect on the total estimated sap flux from Trees W1 and W2, accounting for less than 1% of estimated total flux.

The D/H ratio curves for each of the Toolara trees (Figures 4b and 4c), showed generally similar patterns. There were substantial increases in D/H ratios the day after injection that declined slightly at 2 days. The D/H ratios then increased sharply to reach peak values around 4–5 days after injection before decreasing again. The detailed data of Trees T1 and T2 (Figures 4b and 4c), indicated that the initial increases in D/H ratios after 1 day were a result of increased deuterium concen-

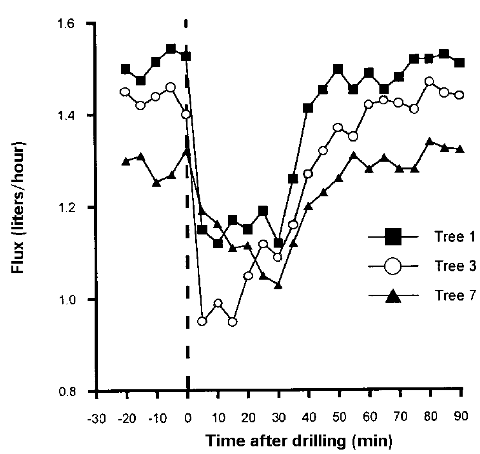

Figure 3. Effect of drilling holes for injection of deuterium on sap flow in Trees T1, T3, and T7 at Toolara.

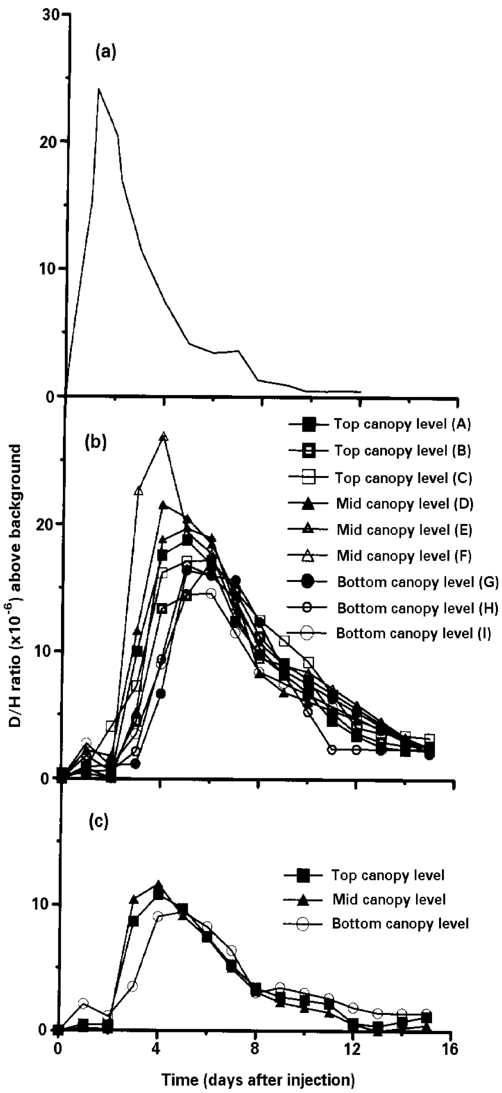

Figure 4. Time course of D_2O concentration (D/H ratio above background) in water transpired by leaves of Tree W1 at Warrill View (a), and Trees T1 (b) and T2 (c) at Toolara over the sampling periods.

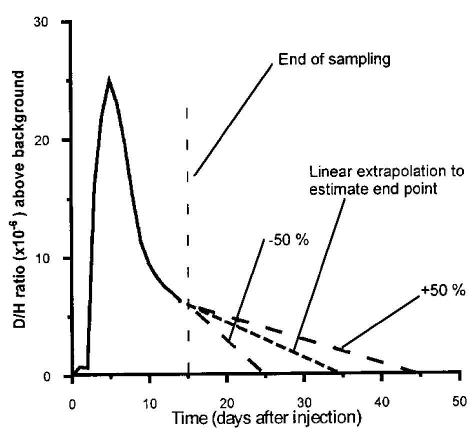

Figure 5. A diagramatic representation of varying end-point estimates with a hypothetical deuterium break through curve.

trations in the lower canopy. We are unable to offer an explanation for what caused these increased concentrations in the lower canopies. In the Toolara trees, there was considerable variation in D/H ratios, both between and within canopy levels (Figures 4b and 4c). The highest peak D/H ratios were detected in the mid-canopy, with the bottom canopy layers showing the lowest peak ratios (after 1 day).

Sap fluxes were calculated separately for each of the nine sampling points in the canopy of Tree T1 (Table 5). (The value in Table 4 is the mean of these nine values.) Sap flux was calculated separately for each canopy layer of Tree T2 (Table 5). The coefficient of variation of sap flux from the nine sample points on Tree T1 was 11%, and 9% between canopy layers. The coefficient of variation of sap flux calculated from the three canopy layers of Tree T2 was 7%.

The D/H ratio in the transpiration samples from the Toolara trees did not return to background values before the end of sampling (15 days after injection) in any of the trees. Linear extrapolation yielded end-point estimates of approximately 35 days after injection for each of the trees except Tree T2 (which had an end-point estimate of 25 days). To assess the sensitivity of calculated flow rates to the projected end points in the Toolara trees, flow rates were calculated based on increasing or decreasing the end-point estimates. Reducing end-point estimates by 50% increased apparent flow rates by 9.8%, on average, whereas increasing estimates by 50 and 100% reduced apparent flow rates by 8.5 and 12.7%, respectively.

Sapwood and heartwood deuterium storage

There were significant concentrations of deuterium in the sapwood and heartwood of the Toolara trees 21 days after injection (Table 6). The deuterium concentration in heartwood was substantially higher than in sapwood.

Sapwood deuterium concentrations at different sample points in Trees SW and HW varied during the spring 1995 experiment, consistent with a pulse of tracer moving through the tree (Figure 6). At 0.5 days after injection, the concentra-

Table 5. Sap flux for nine sample points (*a* to *i*) on Tree T1 and three canopy levels on Tree T2 at Toolara calculated by the deuterium tracing method.

Canopy level	Tree T1 estimated sap flux (l day^{-1})	Tree T2 estimated sap flux (l day^{-1})
Top (*a*)	16.6	
Top (*b*)	17.7	
Top (*c*)	14.0	
Top (mean)	16.1	20.8
Mid (*d*)	15.2	
Mid (*e*)	15.7	
Mid (*f*)	13.5	
Mid (mean)	14.8	23.0
Bottom (*g*)	16.1	
Bottom (*h*)	19.1	
Bottom (*i*)	18.1	
Bottom (mean)	17.8	20.2
Tree mean	16.2 (SD = 1.85; CV = 11%)	21.3 (SD = 1.46; CV = 7%)

tion of deuterium in sapwood about the injection point in Tree SW was much greater than in Tree HW. This was expected because all of the deuterium was injected into the sapwood in Tree SW. At 17 days after injection, however, Trees SW and HW had similar concentrations of deuterium in sapwood. At one day after injection, there was substantially more deuterium in the heartwood of Tree HW, as expected because the deuterium was injected directly into the heartwood of this tree. From Day 4 on, heartwood samples from both trees exhibited substantial amounts of deuterium, although the pattern of accumulation differed between the two trees (Figure 6). By Day 17, deuterium concentrations in the heartwood of Trees SW and HW were approximately equal, indicating that deuterium storage in the heartwood of the trees was the result of both diffusion from the sapwood and (in the case of Tree HW) direct injection of deuterium into the heartwood.

Relationships between flow estimates and canopy and stem dimensions

Consistently strong relationships were observed between leaf area and sapwood area (r^2 = 0.90), leaf area and DBH (r^2 = 0.93), and sapwood area and DBH (r^2 = 0.90) across both sites (cf. Waring and Schlesinger 1985, Olbrich et al. 1993). There were strong correlations between sap flux estimated by the deuterium tracing method and leaf area (r^2 = 0.90), sapwood area (r^2 = 0.93), and DBH (r^2 = 0.91). The relationship between sap flux estimated by the heat pulse method and these parameters was not as strong (r^2 = 0.56, r^2 = 0.84, r^2 = 0.78, respectively) primarily because Tree T2 had a lower sap flux relative to its size (i.e., leaf area, sapwood area and DBH) than the other trees. This suggests that there were problems with the heat pulse estimated flux for this tree. When the results from Tree T2 were excluded from the regression analyses, the relationships between sap flux estimated by the heat pulse method and the parameters leaf area, sapwood area, and DBH were similar to those obtained with the deuterium tracing method (r^2 = 0.93, 0.95 and 0.94, respectively).

Comparison of flow estimates

The deuterium tracing method gave estimates of sap flow flux between 11% (W1) and 18% (W2) greater than the heat pulse method for the Warrill View trees, and between 113% (T2) and 12% (T1) greater than the heat pulse results for the Toolara trees (Table 4). The relationship between the heat pulse and deuterium estimates of sap flux for the two Warrill View trees and six Toolara trees (i.e., Tree T2 not included) had an r^2 = 0.98 ($y = 1.2\ (\pm\ 0.04)x$).

Table 6. Residual deuterium concentrations above background in Toolara trees 21 days after injection (background D/H ratio was 156.4 ± 0.19×10^{-6}).

Tree	Sapwood concentration (D/H ratio $\times 10^{-6}$)	Heartwood concentration (D/H ratio $\times 10^{-6}$)
T1	5.8	62.9
T2	2.0	9.1
T3	19.8	75.8
T4	5.8	33.5
T5	23.1	82.0
T6	8.1	22.1
T7	13.4	71.8

Discussion

The deuterium tracing method gave 10 to 43% higher estimates (excluding Tree T2) than the heat pulse method for all experimental trees (Table 4). In contrast, Dye et al. (1992) reported that deuterium tracing estimates of sap flux of two *E. grandis* trees were 26 and 7% less than heat pulse estimates of sap flux.

Although the heat pulse technique has previously been shown to provide generally accurate estimates of sap flux in *Eucalyptus* trees (e.g., Dunn and Connor 1993) including *E. grandis* (Olbrich 1991), errors associated with estimating individual tree water use (including errors in sap flux measurements, and errors associated with the integration of sap flux over the conducting wood area) can be as high as 38% (Hatton et al. 1995). With the exception of Tree T2, this degree of error could account for most of the observed difference between the two methods. For example, sap flux estimates increase by 10 (Tree T3) to 21% (Tree T7) in response to a 10% increase in wound size.

Potential errors associated with the deuterium tracing method, particularly the estimate of when the deuterium concentration returned to background values, may also have accounted for some of the differences in sap flux estimated by the two methods. We found significant storage of deuterium in the heartwood 21 days after injection (Table 6). There are a number of possible reasons why this occurred. The most likely explanation is that, after the deuterium was introduced into the trees and taken up in the xylem flow, diffusion occurred from the sapwood, through the rays and axial parenchyma, into the heartwood along a deuterium concentration gradient. The diffusion of deuterium into the heartwood would act to slow the complete release of deuterium from the system. Once the main pulse of deuterium had passed through the sapwood, the portion of deuterium that had diffused into the heartwood would slowly diffuse back into the conducting sapwood. The amount of deuterium remaining in the tree after 21 days suggests that the end-point estimates used in the calculation of sap fluxes were conservative, leading to overestimates of sap flux.

The deuterium tracing technique may also be prone to errors as a result of drilling holes for deuterium injection (Dye et al. 1992). Other studies have found that holes drilled in the sapwood of trees caused lateral disruption of water transport, and cavitation of water columns in vessels near the holes (e.g., Doley and Grieve 1966). In our study, however, any disruption to xylem flow paths caused by the deuterium injection holes did not significantly alter measured sap flow. Sap flow measured with the heat pulse units quickly stabilized at rates similar to pre-injection rates in the three trees (T1, T3 and T7) that showed some variation in flow before and after drilling injection holes. Transpiration may be little affected by minor damage to xylem vessels because the interconnected nature of

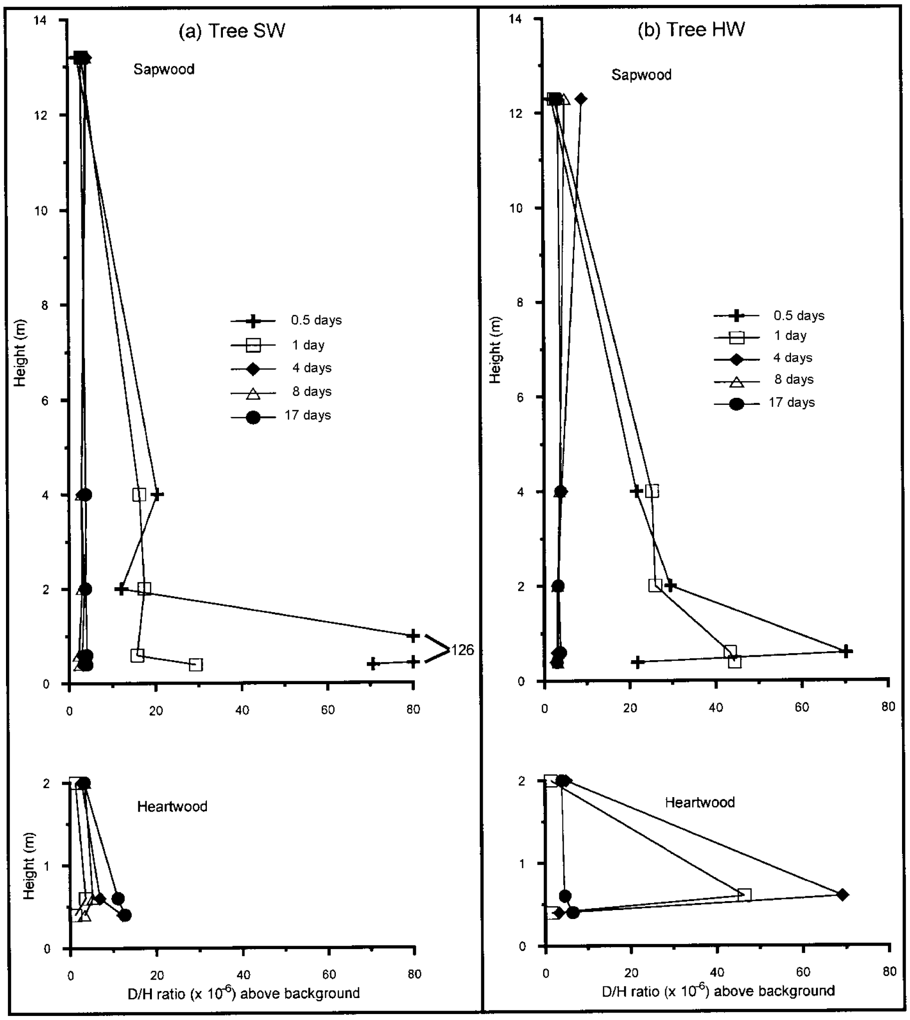

Figure 6. Time course of D_2O concentration (D/H ratio above background) in the sapwood and heartwood of Tree SW (a) and Tree HW (b). There was no heartwood development at 4 m in either tree

xylem vessels offers numerous alternative pathways for water around the point of damage (Slatyer 1967).

The variation in deuterium concentration between and within canopy levels (illustrated in Figures 4b and 4c) may be a result of specific portions of the sapwood leading to different parts of the canopy. By using many (up to 32) small holes for injecting the deuterium, it was assumed that mixing of the tracer within the tree would be improved (see Kline et al. 1970), reducing the chance of preferential flow from injection holes to specific points in the canopy. The coefficient of variation of sap flux calculated from the different sample points on Tree T1 (11%) was significantly lower than in deuterium tracing studies using six injection holes (24%; Dugas et al. 1993). The coefficients of variation of the sap flux from the different canopy layers in Trees T1 and T2 (9 and 7%) were also markedly lower than those obtained between canopy layers in other studies (e.g., 11 and 15%; Calder 1986). Another factor that may have influenced the variation in deuterium concentrations both within and between canopy layers is that different sides and positions of a single tree crown receive different radiation loads and are under differing evaporative demands, resulting in variations in stomatal behavior and transpiration.

This study has highlighted both the difficulty of determining residual tracer storage in trees when using the deuterium tracing method, and the role heartwood plays in storing deuterium. We conclude that the deuterium tracing technique is more suited to younger trees with no (or limited) heartwood, and that a knowledge of tree structure, especially the thickness of the conducting sapwood, is critical for the successful application of the technique.

Another aim of this study was to compare the heat pulse and deuterium tracing methods of estimating tree water use at the scale of the individual plant. Quantitative information on water use of individual trees is often used as a basis for scaling up to stands or catchments in order to assess transpiration rates. In terms of the accuracy of scaling individual-tree water-use information to larger areas, Hatton et al. (1995) have demonstrated that increasing the sample size on which the scaling is based increases the degree of confidence of the scaled-up estimates. However, if large stands of trees are the subject of study, the sample size requirements may quickly become prohibitively expensive. The deuterium tracing technique offers a cheaper, though more labor intensive way of obtaining estimates of large sample sizes than measuring sap flux in many trees by the heat pulse method. The possibility of using both techniques simultaneously also exists, though this would be best in stands where large differences in sap flow between trees exist (e.g., because of a range of tree sizes), so that relative differences in sap flux estimates between the methods are small compared to the range of sap flow rates.

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