Test of the deuterium tracer method used in the determination of transpiration rates in trees and a comparison of the transpiration rate of *Acacia mearnsii* and *Leucospermum conocarpodendron*

Honours Project

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Abstract

The accuracy of the deuterium tracing method was determined, using a gravimetric technique to determine water loss. The deuterium tracing technique was then employed to determine and compare the transpiration rate of *Leucospermum conocarpodendron*, a Proteaceae species that is indigenous to the Western Cape, and *Acacia mearnsii*, an invasive alien. *L. conocarpodendron* was found to transpire significantly less than *A. mearnsii* on a per unit leaf area basis (0.97 l. $m^{-2}.d^{-1}$ versus 1.49 l. $m^{-2}.d^{-1}$ respectively). This information has implications for managing water catchments with regard to invasive aliens, to ensure an optimal supply of water in a country where water is a scarce commodity.

Key words: Transpiration rate, deuterium tracer method, gravimetric method, *Leucospermum conocarpodendron*, *Acacia mearnsii*, leaf area, water resources

Introduction

The deuterium tracing technique, whereby the transpiration rate of trees can be determined, has been recently used by Calder et al. (1986); Calder et al. (1992); Dye, Olbrich & Calder (1992); Dugas, Wallace, Allen, & Roberts (1993); Calder (1998) and Kalma, Thornburn & Dunn. (in press). This method has been compared to the heat pulse method (Kalma et al. 1992, Dye et al. 1992) for verification but has not been rigorously tested using gravimetric methods. Calder et al. (1992) used excavated trees that were put into water containers and the transpiration rate then measured gravimetrically. This may however not be a realistic way to verify the deuterium tracer method as many of the field conditions, such as soil water potential and water stress, were not taken into account. Dugas et al. (1993) tested this method gravimetrically using potted *Eucalyptus gunnii* trees and found the transpiration rate using the deuterium method was greater than the amount measured gravimetrically. The slope for transpiration measured gravimetrically versus transpiration measured using the deuterium method was 1.3 with a root mean squared difference of 1.0 kg per tree. This suggests that the deuterium tracer method tends to overestimate the transpiration rate.

In this experiment the deuterium tracing technique has been further verified gravimetrically using a tree indigenous to the Western Cape, namely *Rapanea melanophloeos*. The technique was then employed to determine and compare the transpiration rates of *Leucospermum conocarpodendron*, an indigenous Proteaceae species and *Acacia mearnsii*, an invasive alien species. Bergh (1998) reported on a similar analysis of the water uses of *Hakea* species and *Protea* species.

There is little information available on the mechanisms involved in the loss of water from catchments. Although various experiments have been set up to measure the rate of runoff from a catchment Beven & Wood (1983), little has been done on the effects of vegetation on water cycling in catchments. Wicht (1971) suggested that vegetation plays a major role in the hydro-dynamics of such an area. We assume that *L. conocarpodendron* trees utilise less water on a per unit leaf area basis than *A. mearnsii* trees as they appear to be slower growing and well adapted to areas that experience summer drought. Therefore a catchment area infested with *A. mearnsii* trees will deliver less water than a catchment populated with indigenous species. It has been shown, using water catchment experiments that a catchment area that is infested with alien species delivers less water than if it were managed for species such as *L. conocarpodendron*. This is however only done on a catchment scale, not on the individual tree scale and "can easily lead to erroneous results" (Dye, Olbrich & Everson 1998).

The objective of this study was firstly to further verify the deuterium tracer technique and then to determine the water use of *L. conocarpodendron* trees and compare it to that of *A. mearnsii* trees commonly found in catchment areas.

Site description

The accuracy of the deuterium method was tested using a scale in the glass-house of the botany department at the University of Cape Town. The field analysis was done at Camps Bay (34° 57' 34" S, 18° 23' 15" E). The field site was on the southern bank of the Blinkwater stream, *c.* 50 m above the stream and 140 m above sea level. The vegetation is a mixture of scrub forest and fynbos and is infested with alien species.

The data were collected after the first rains, from 11 May until 26 May. The site is rocky and has soils of varying depths. The soil is derived from sandstone, has an apedal structure and is well drained. The site is subjected to a mediterranean climate.

Method

The method used was similar to that described by Calder et al. (1986). The transpiration rates of *Acacia mearnsii* and *Leucospermum conocarpodendron* were measured using deuterium oxide (D_2O) with a minimum isotopic purity of 99.8%, as a tracer in the 'total counts' and averaged-time methods, as described by Calder (1991). A known amount of deuterium was injected into the base of the tree where it mixes with the water that moves up the tree in the sapwood. Samples of this mixture were taken from the water that transpired from the leaves on selected branches. These samples represent the integrated concentration of the deuterium moving past fixed locations up the tree during each 24-hour interval. The concentration of the deuterium is dependent on the amount of water that it mixes with. This in turn is dependent on how much water the tree transpires. For each of the species used, the samples taken from each branch were pooled on a daily basis.

The pooling of samples is done by taking equal amounts from each sample collected, using a micropipette, with a clean tip for each sample to prevent cross-contamination. Once all the samples were collected at the end of the experiment they were pooled for each tree to get an average for the duration of the experiment. This is known as the averaged-time method for which equation 1 is applied to determine the transpiration rate for the duration of the experiment.

$$F = \frac{M}{T.\Delta t.C_m} \quad \text{.........................} (1)$$

F is the weighted mean flow rate, M is the amount of deuterium injected in litres, T is the time increment during which the last sample was taken, Δt is the time interval between taking samples and Cm represents the mean of the daily concentrations i.e. the concentration of the pooled sample $[(^2H/^1H)_{sample}]$.

The output value from the mass spectrometer is given as δ^2H_{VSMOW} where

$$\delta^2H = ((^2H/^1H)_{sample})/(^2H/^1H)_{reference}) - 1) \times 1000 ‰ \text{ VSMOW}$$

$$= ((^2H/^1H)_{sample})/0.0001558) - 1) \times 1000 ‰ \text{ VSMOW}$$

$$\therefore (^2H/^1H)_{sample} = (\delta^2H/1000 + 1) \times 0.000158$$

A tree from each species was selected to determine the transpiration using the 'total counts' method. For this method the daily-pooled samples are analysed separately and equation 2 is used to determine the transpiration.

$$F = \frac{M}{\sum_{i=1}^{i=Time} C_i t_i} \quad \text{.........................} (2)$$

M is the amount of the tracer injected in litres, C_i is the daily concentration ($^2H/^1H$), t_i is the duration of the *i*th time increment and Time is the time that it takes for at least 90% of the tracer to pass through the tree (Dugas et al., 1993):

Gravimetric versus tracer method

The accuracy of the deuterium method was tested on a potted *Rapanea melanophloeos*. The specifications of the tree are listed in Table 1. The tree was planted in a sandy-loam potting mixture in a standard plastic plant pot with a top diameter of 43cm. The potting mixture was saturated with water and the excess water was allowed to drain off. The pot was then sealed at the base and put into a plastic bag that was tied closed around the base of the tree to prevent water loss from the soil due to evaporation. The entire unit consisting of the tree, the soil, the pot and the bag was then balanced on a scale with counter-weights. Every 24 hours a measured amount of water was added to the soil until the mass of the unit was equal to its original mass when the soil was saturated, at which stage the scale was considered as 'balanced'. The amount of water transpired each day could then be calculated.

Table 1 Specifications of the trees used and the amount of deuterium (D_2O) that was injected into the base of the trees. The diameter of the tree was measured 30 cm above the soil.

Tree no.	Stem diameter (cm)	Tree height (m)	Height of lowest branch (m)	Number of branches sampled	Canopy diameter (m)	D_2O (ml)
Rapanea melanophloeos						
1	3.20	2.00	0.59	4		1.10
Acacia mearnsii						
1	2.93	2.85	1.70	1		0.40
2	4.33	2.84	0.93	2		0.75
3	5.03	3.68	0.44	3		0.93
4	9.23	4.90	0.62	3		1.60
5	4.84	3.16	0.50	3		0.80
Leucospermum conocarpodendron						
1	5.60	1.20	0.34	2	0.70	0.70
2	12.70	1.92	0.40	3	1.90	1.60
3	19.70	2.60	0.88	4	2.90	2.00
4	7.40	1.10	0.25	2	0.80	0.75
5	25.50	2.82	0.40	4	3.20	2.20

Figure 1. Experimental set-up for testing the accuracy of the deuterium tracer method on *Rapanea melanophloeos* using a gravimetric technique.

The scale was designed using a 2m length of rectangle cross-section mild steel hinged with two pillow bearings on either side and held by a 25 mm diameter stainless steel shaft. The mass of the potted tree when the soil was saturated was 65 kg and the scale was sensitive to 10 g. Two heater-fans, placed on opposite sides of the tree and a spot light, were used to increase the temperature and decrease the humidity around the tree, to increase the rate of evapo-transpiration, as the glass-house was humid and cold, being winter. The set-up without the fan-heaters can be seen in Figure 1.

The relative humidity and temperature was measured each day and regulated to ensure a suitable microclimate for *R. melanophloeos* to transpire adequately. This was done by manipulating the heater-fans and spotlight.

The background reading of the deuterium concentration was taken before the tree was injected with deuterium by placing four clear plastic bags on the tree to capture the water that transpired from the tree. These bags were 62.0 X 40.5 cm and enclosed at least six leaves. The leaf samples had to represent the canopy, therefore two bags were placed on the lower branches and two upper branches, as can be seen in Figure 1. The average concentration of the deuterium in the condensate that was collected in the bags was then measured using the 'total counts' method as described below. Once the potting soil was saturated and the unit balanced, the tree was injected with 1.1 ml of deuterium that was evenly divided by volume amongst five holes of 4 mm diameter and *c.* 30 mm deep. These holes were positioned equidistantly around the circumference of the stem at 10 cm above the soil. They were drilled downwards at 45° to the horizontal to contain the deuterium. The amount of deuterium that was injected into the tree was based on an estimate using the flow rate of *Acacia mearnsii* trees as the maximum possible flow rate. The holes were then plugged with Brummer waterproof stopping exterior (wood putty) to prevent evaporation and leakage of the deuterium. The trees were injected at 15h00, as this is when the trees are in a state of water deficit and can therefore take the tracer up more quickly into the sapwood (Calder, 1998 pers. comm.).

At 17h00 each day the bags were replaced and 1.5 ml of the condensate was collected from each bag, by dipping an eppendorf into the bag. It is preferable that the samples are taken at the same time each day to simplify calculations. Equal quantities of the samples taken from the bags on each tree were pooled into one sample for analysis. This will provide a representation of the water-use of the tree as a whole. The samples for each day are also pooled separately to determine the break-through curve. This is a curve that represents the average concentration of the deuterium in the tree for each day.

The samples taken from the bags were analysed by determining the concentration of the deuterium using a Finnegan MAT 252 dual inlet mass spectrometer.

Transpiration rate in field

A range of size classes of an alien species and an indigenous species, namely *Acacia mearnsii* and *Leucospermum conocarpodendron* respectively, were selected. The specifications for these trees and the number of bags used are recorded in Table 1. The trees were treated using the same methods as used for *R. melanophloeos* above. Not more than 20 % of the crown was covered with plastic bags, as the microclimate in the bags could alter the rate of evapo-transpiration of the tree if the bags occupied a significant portion of the canopy. The bags must also not be exposed to the air for too long whilst taking the samples, to prevent loss of water through evaporation. This would result in fractionation of the condensate. Fractionation occurs when the lighter water molecules evaporate, leaving behind the heavier deuterium molecules.

Once the condensate for all the trees was collected, the total leaf surface area was determined. The leaf area for the *L. conocarpodendron* trees were determined by randomly selecting a sample of three trees, measuring the diameter of 34 branches, *c.* 2 cm from the base, and feeding the leaves from each branch through a Li-Cor LI-3000 portable leaf area meter. The leaf area was then correlated with the diameter of the branch. The branches of the trees used in the experiment were then measured and the leaf area was extrapolated from an equation derived from the correlation determined above.

The leaf areas for the *A. mearnsii* trees were determined in a similar way except that the area was correlated with the dry mass of the leaves. Branches were randomly selected from adjacent trees. The leaf area was measured using the area meter and then correlated with the dry mass. All the leaves were then removed from the trees in the experiment, oven dried and the area calculated from the equation determined in the correlation above. All leaf material was oven dried at 70°C for 24 hours. The branch diameter was also measured and correlated with the calculated leaf area.

The transpiration rate per unit leaf area was then calculated for each tree, using the transpiration results from equation 1, and the leaf area results. The statistics were performed using Statistica 98 and the spreadsheet package used was Excel (Office 97).

Results

Gravimetric versus tracer method

The deuterium concentration in *Rapanea melanophloeos* reached its peak (0.037 % above background reading) on the third day and then tailed off to near background concentration (0.00059 % above background reading) after ten days (Fig. 2). Applying the averaged-time method (eqn. 1), the flow rate is calculated to be *c.* 0.95 l. d^{-1}. As expected, a similar result of *c.* 0.96 l. d^{-1} was obtained with the 'total counts' method (eqn. 2). The gravimetric method, however, estimates the transpiration to be *c.* 0.64 l. d^{-1}. This constitutes a 33 % difference between the deuterium tracer method and the gravimetric method.

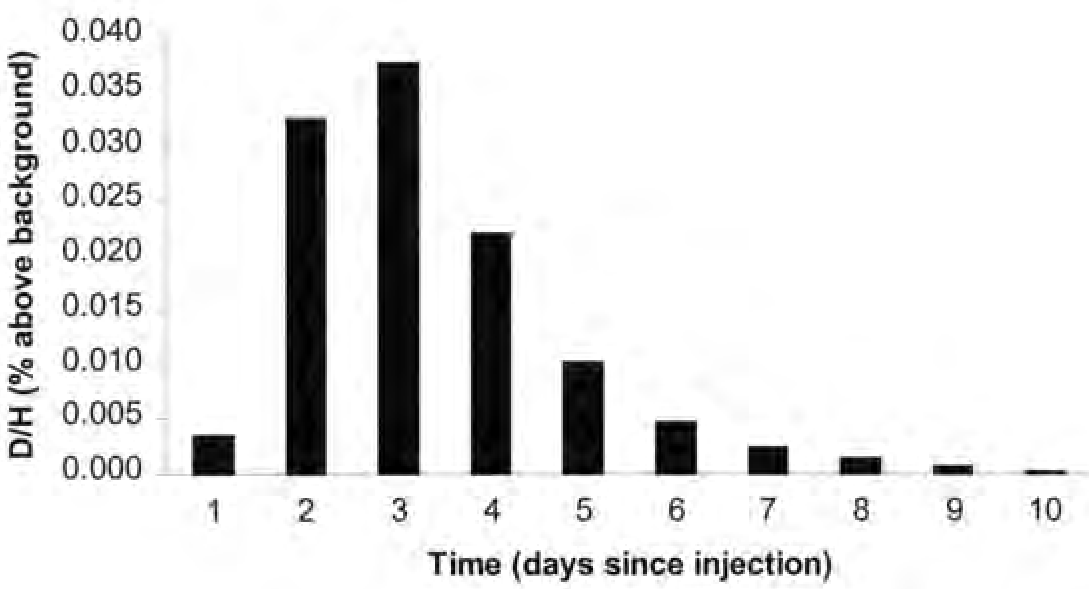

Figure 2. The concentration of deuterium above background in *Rapanea melanophloeos* for each consecutive day after the tree was injected with deuterium

Transpiration rate in field

The concentration of deuterium reached its maximum in the branches of *A. mearnsii* two days after the tree was injected and then tailed off (Fig. 3). There was however, still 0.00011 % deuterium left in the tree when the experiment was terminated after 11 days. The tail was therefore projected onto the x-axis at Day 14 where the deuterium concentration would be zero as described by Dye, Olbrich & Calder (1992). The missing deuterium values were then interpolated and incorporated into equation 2 to determine the rate of evapo-transpiration. The wind blew strongly on Day 9 resulting in the trees not transpiring. There was therefore not enough condensate in the bags to collect. The bags were therefore left on the trees until the following day, although as a result of this, the concentration was almost twice as high on Day 10 as it should have been. Day 9 was still included in the analysis and therefore corrected for the high concentration measured on day 10.

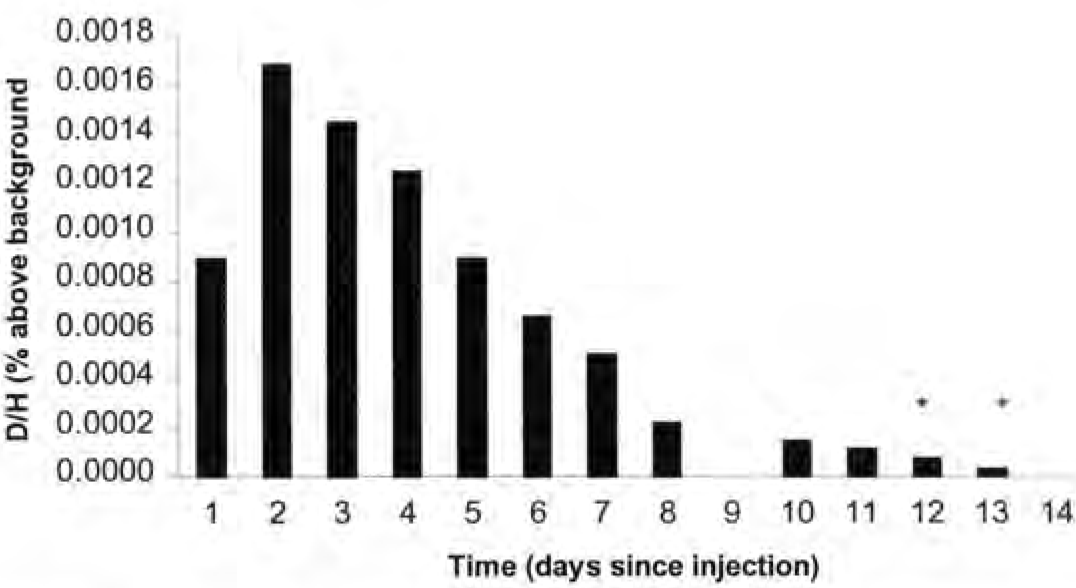

Figure 3. The concentration of deuterium above background in *Acacia mearnsii*, tree no. 3, for each consecutive day after the tree was injected with deuterium. * indicates the interpolated values. See text for explanation.

L. conocarpodendron, tree no. 5 was selected for the daily analysis (Fig. 4). The same circumstances and predicaments applied to the *L. conocarpodendron* trees as for the *A. mearnsii* trees above. When the experiment was terminated there was still 0.00016 % deuterium left in the tree. The tail was projected as above and reached the x-axis before Day 14. The results for the concentration of deuterium during the first 2 days are peculiar in that they are lower than the background reading. The concentration peaks at Day 5 and only then starts to tail off.

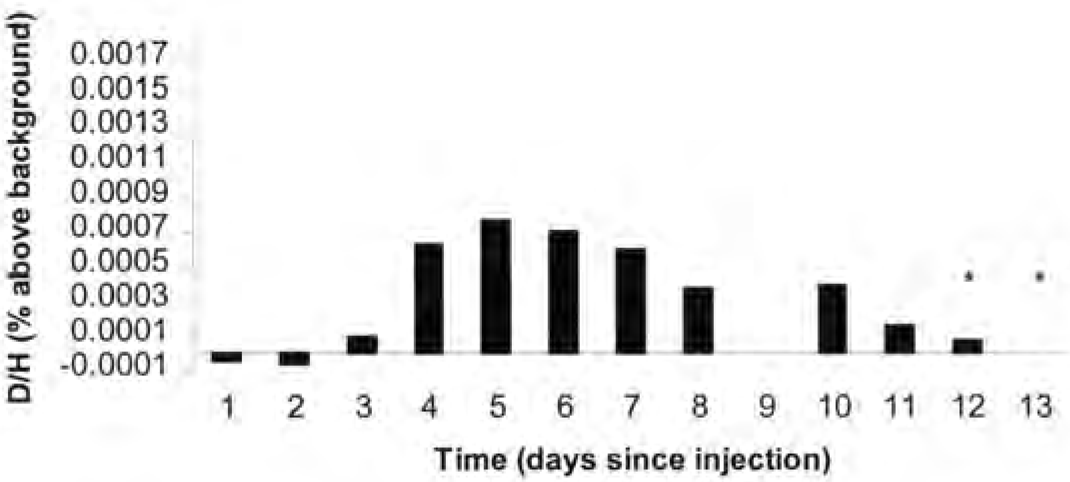

Figure 4. The concentration of deuterium above background in *L. conocarpodendron*, tree no. 3, for each consecutive day after the tree was injected with deuterium. * indicates the interpolated values. See text for explanation.

The calculations to determine the flow rates of *L. conocarpodendron* and *A. mearnsii* using the 'total counts' method are shown in Table 2 and the arithmetic below. The flow rates using the averaged-time method for the respective species are shown in Table 3. From these results it appears that *L. conocarpodendron* has a substantially greater flow rate than *A. mearnsii* on a per tree basis, but not on a per unit leaf area or per unit stem area basis.

Table 2 Calculations of the denominator for Equation 2 to determine the flow rate in *L. conocarpodendron* and *A. mearnsii* using the 'total counts' method. The values with '*' are the interpolated values

δ^2H	D/H	BG δ^2H	BG D/H	C_i	C_i (%)	ti d	C_i X t_i
Leucospermum conocarpodendron, tree no. 5							
-1	0.000156	2	0.000156	-4.674E-07	-0.00005	1.00	-0.0000005
-2	0.000155	2	0.000156	-6.232E-07	-0.00006	0.99	-0.0000006
8	0.000157	2	0.000156	9.348E-07	0.00009	0.99	0.0000009
42	0.000162	2	0.000156	6.232E-06	0.00062	1.02	0.0000064
51	0.000164	2	0.000156	7.6342E-06	0.00076	1.00	0.0000076
47	0.000163	2	0.000156	7.011E-06	0.00070	1.00	0.0000070
40	0.000162	2	0.000156	5.9204E-06	0.00059	0.99	0.0000059
26	0.000160	2	0.000156	3.7392E-06	0.00037	1.00	0.0000037
27	0.000160	2	0.000156	3.895E-06	0.00039	2.01	0.0000078
12	0.000158	2	0.000156	1.558E-06	0.00016	1.00	0.0000016
					0.00007*	1.00	0.0000007
					0.00000*	1.00	0.0000000
						$\sum C_i t_i$ =	0.0000416
Acacia mearnsii, tree no.4							
60	0.000165	3	0.000156	8.8806E-06	0.00089	1.00	0.0000089
111	0.000173	3	0.000156	1.6826E-05	0.00168	0.99	0.0000167
96	0.000171	3	0.000156	1.4489E-05	0.00145	0.99	0.0000143
83	0.000169	3	0.000156	1.2464E-05	0.00125	1.02	0.0000127
60	0.000165	3	0.000156	8.8806E-06	0.00089	1.00	0.0000089
45	0.000163	3	0.000156	6.5436E-06	0.00065	1.00	0.0000065
35	0.000161	3	0.000156	4.9856E-06	0.00050	0.99	0.0000049
17	0.000158	3	0.000156	2.1812E-06	0.00022	1.00	0.0000022
12	0.000158	3	0.000156	1.4022E-06	0.00014	2.01	0.0000028
10	0.000157	3	0.000156	1.0906E-06	0.00011	1.00	0.0000011
					0.00007*	1.00	0.0000007
					0.00003*	1.00	0.0000003
					0.00000*	1.00	0.0000000
						$\sum C_i t_i$ =	0.0000800

'Total counts' method:

L. conocarpodendron: $F = M/\sum C_i.t_i = 0.002$ l / 0.0000416. d = 52.86 l. d^{-1}

A. mearnsii: F = 0.0016 l / 0.0000800. d = 19.99 l. d^{-1}

Table 3 The flow rates for the respective trees of *Leucospermum conocarpodendron* and *Acacia mearnsii* using the averaged time method ($F = M / T.\Delta t.Cm$).

Leucospermum conocarpodendron		*Acacia mearnsii*	
Tree no.	Flow rate (F) $l.d^{-1}$	Tree no.	Flow rate (F) $l.d^{-1}$
1	3.52	1	2.59
2	13.73	2	4.76
3	25.37	3	4.80
4	2.59	4	18.67
5	58.35	5	10.61

Determination of leaf area

The equation of the straight-line graph of log diameter versus log leaf area of *L. conocarpodendron* (Fig. 5 & 6) is $y = 2.55x - 0.005$, $r^2 = 0.74$, $n = 92$ and of the log dry mass versus log leaf area of *A. mearnsii* (Appendix 1) is $y = 1.03x + 1.74$, $r^2 = 0.97$, $n = 75$. These equations were used to determine the leaf area for the respective *L. conocarpodendron* and *A. mearnsii* trees (Table 4). The leaf areas were also calculated for branches for which the leaf area was measured. The resultant equation for measured area versus calculated area was $y = 0.973X + 0.089$, $r2 = 0.97$and the correlation coefficient was 0.98.

Table 4 Leaf area and basal stem area for *L. conocarpodendron* and *A. mearnsii* trees

	Leucospermum conocarpodendron		*A. mearnsii*	
Tree no.	Leaf area m^2	Stem area cm^2	Leaf area m^2	Stem area cm^2
1	2.58	24.62	1.26	6.60
2	15.37	126.61	3.49	14.51
3	30.91	304.65	2.90	19.63
4	4.74	42.99	17.49	66.89
5	48.44	510.45	7.96	18.38

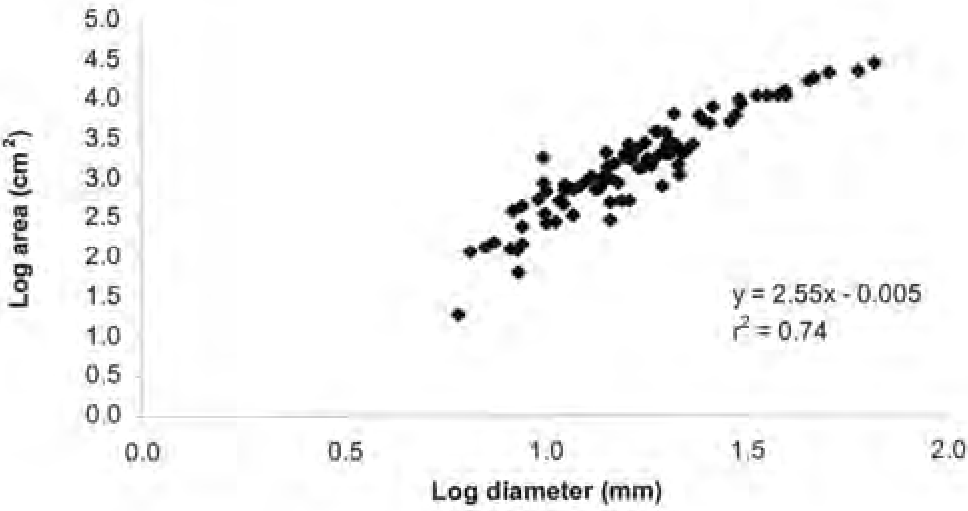

Figure 5. The log area of the leaves of *L. conocarpodendron* plotted against the log diameter of the branches holding each bunch of leaves.

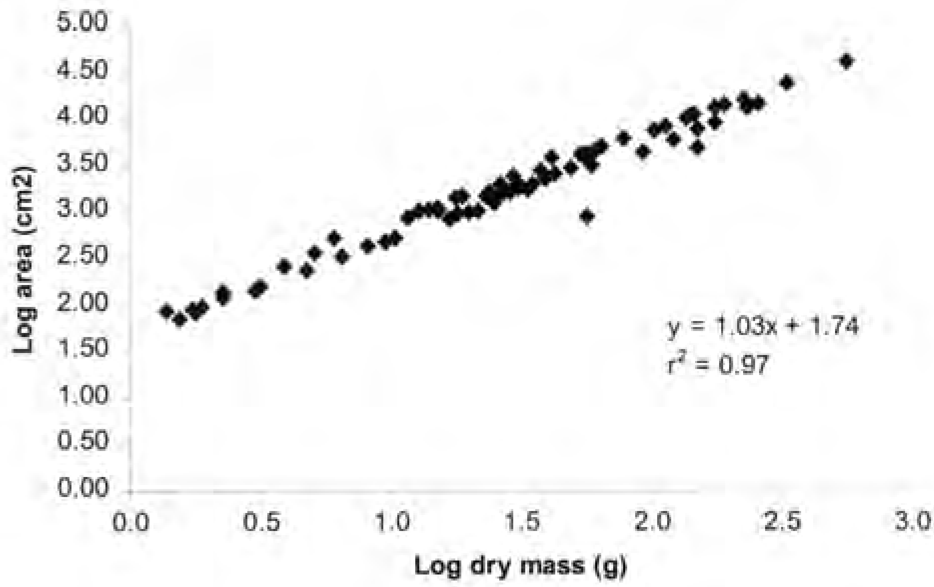

Figure 6. Log area of the leaves of *A. mearnsii* plotted against the log dry mass of the leaves

Although *L. conocarpodendron* has a higher flow rate on a per tree basis, it has a significantly lower flow rate on a per unit leaf area and stem area basis (Mann-Whitney U test, $P < 0.005$, d.f = 9) (Table 5).

Table 5 Transpiration rates for *L. conocarpodendron* and *A. mearnsii* on per unit leaf area and per unit stem area basis. Different letters indicate statistically significant differences at the $P < 0.005$ level (Mann-Whitney U test, calculated by hand).

Species & tree no.	Flow rate per unit leaf area $l. m^{-2}. d^{-1}$	Flow rate per unit stem area $l. cm^{-2}. d^{-1}$
L. conocarpodendron		
1	1.37	0.14
2	0.89	0.11
3	0.82	0.08
4	0.55	0.06
5	1.20	0.11
Mean	0.97^{a}	0.10^{c}
A. mearnsii		
1	2.05	0.39
2	1.36	0.32
3	1.66	0.24
4	1.07	0.28
5	1.33	0.58
Mean	1.49^{b}	0.36^{d}

The Pearsons Correlation Coefficient for basal stem area and water flow was *c.* 0.98 for *L. conocarpodendron* and *c.* 0.93 for *A. mearnsii* ($P < 0.05$, d.f. = 4) with $r^2 = 0.97$ and 0.87 respectively. With the exception of an outlier, there also appears to be a general increase in the transpiration rate per unit leaf area for *L. conocarpodendron* with increase in tree size as determined by the diameter near the base of the tree (Fig. 7). *A. mearnsii* shows an opposite trend (Fig. 8), as the transpiration rate increases with increasing basal stem area.

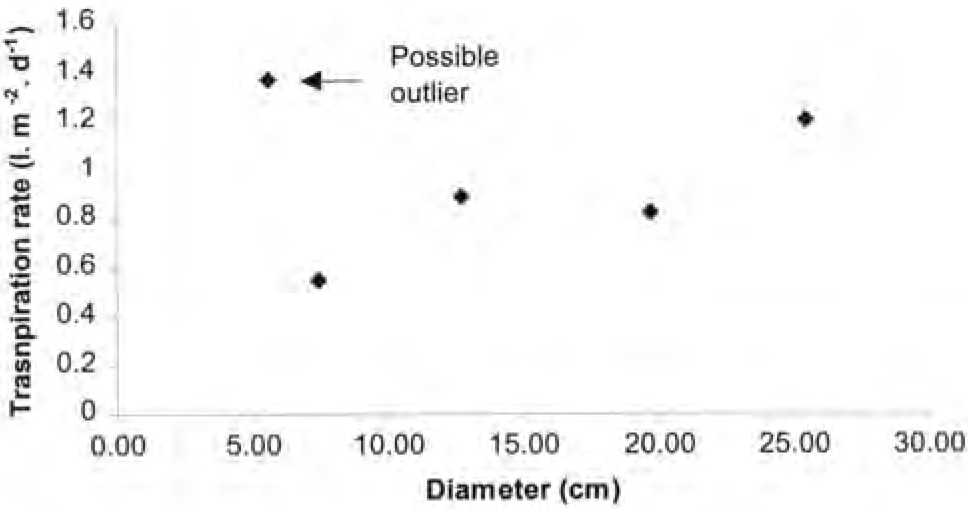

Figure 7. Basal stem diameter versus transpiration rate per unit leaf area of the five *L. conocarpodendron* trees.

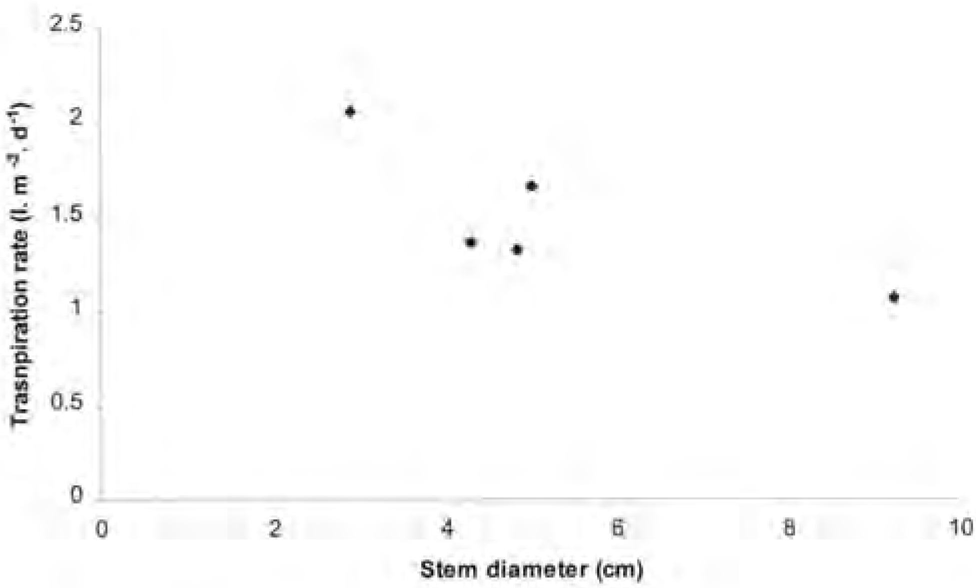

Figure 8. Basal stem diameter versus transpiration rate per unit leaf area of the five *A. mearnsii* trees.

Discussion

Gravimetric versus tracer method

There appears to be a significant difference in the transpiration rates estimated between the deuterium tracer method and the gravimetric method. The transpiration rate as determined by the deuterium tracer method, appeared to be at least 33 % more than that measured by the gravimetric method. Dugas et al. (1993) found similar results for *Eucalyptus gunnii* trees. If the scale was not calibrated correctly it would have overestimated the rate of flow because it would have required extra water to overcome the inertia of the system. During calibration, however, the maximum force required to overcome the resistance was *c.* 0.1 N exerted at the center of gravity of the tree. This amounts to *c.* 10 ml of water. The tree may also have shifted slightly towards the distal end of the scale, thereby underestimating the amount of water used by the tree. This, however, is unlikely. If there were any error pertaining to the daily gravimetric measurements it would have been compounded by the end of the 10 days. As it is not necessary to determine how much the tree transpires each day, it would have been more accurate to measure the amount of water transpired at the end of the experiment. It would also have been preferable to have more bags on the tree, but there is the risk of reducing the transpiration too much.

There was also still some deuterium left in the water flowing through the sapwood when the experiment was terminated. If the experiment were continued until all the deuterium in this water had returned to the background concentration, the measured flow rate would have decreased further, resulting in the system being more accurate. One of the requirements for this technique to work is that the deuterium concentration in the tree must return to the background level (Calder 1998).

There are various possible reasons why the deuterium tracer method, as employed in this experiment, would over-estimate the transpiration rate of trees. (1) Some of the deuterium may have leaked out of the hole from where it was injected as the hole was filled up to the inside of the bark to minimize embolisms. Plugging the hole may also have squeezed some out. (2) Some of the hydrogen atoms that are bound to various elements such as carbon in the wood are exchangeable (Luvall & Murphy 1982, Calder 1991) and could therefore temporarily bind the hydrogen atoms of the deuterium. (3) The water in the heartwood does not flow up the stem as in the sapwood. This water interchanges with deuterium and therefore some of the deuterium injected is partially removed form the system (Kalma et al. in press) and would result in a lower concentration of the deuterium in the water flowing through the sapwood. These effects would, however, be consistent and could therefore be accounted for.

There appears to be different flow rates within the canopy of a tree (Luvall & Murphy 1982). Calder et al. (1986) and Dugas et al. (1983) found that there were differences in the concentration of deuterium found at various heights. This may be due to certain conduits conducting more water than others (Calder et al. 1986). Kalma, Thornburn & Dunn (in press), however, found little variation. In my experiment the bags were nevertheless positioned in such a way as to sample an average concentration for the whole canopy and therefore provide a mean transpiration rate for the tree.

These bags collect an integrated sample over the time period for which the bags are on the tree. The samples need to be collected each day to ensure that the concentration of the deuterium is known when it reaches its peak, as this is important for calculating the transpiration rate.

Although the transpiration rate in the bags is lower, due to the higher humidity, it does not affect this technique, because the bags are used only as sampling points. It serves a similar purpose as using a syringe to draw the water out of the tree, but in this case the water is extracted continuously over a 24-hour period. It is also for this reason that the bags used for sampling should cover less than 20 % of the canopy, otherwise the transpiration rate of the tree could be impaired.

Despite the difference measured between the deuterium tracing method and the gravimetric method the deuterium tracing method still appears suitable for use in the determination of transpiration rates in trees. It has a dual purpose as it measures actual amount of water transpired and can be used to compare the relative transpiration rates of different species. There should, however be more experimentation using gravimetric methods to determine if different species have a differential effect on the flow rate as measured using the deuterium tracer method.

The transpiration rates of A. mearnsii and L. conocarpodendron

The results suggest that although *L. conocarpodendron* trees used in the experiment transpired more water than the *A. mearnsii* trees, the transpiration per unit leaf area and basal stem area were significantly less. The *A. mearnsii* trees furthermore appear to transpire substantially more water than the *Eucalyptus tereticornis* trees of similar basal stem area, measured by Calder (1998).

The strong correlation and regression values for basal stem area versus flow rate for *L. conocarpodendron* and *A. mearnsii* species support the suggestion that the deuterium tracer method is suitable for flow measurements in the field. A correction factor may however be necessary. This would need to be determined using a more rigorous approach.

The larger *A. mearnsii* trees appear to transpire less water on a per unit leaf area basis than the smaller trees. This may be indicative of a greater root-shoot ratio of the seedlings. *A. mearnsii* may have adopted a drought-avoidance strategy by developing a large root system that could tap into the ground water before a severe drought. Once it reaches the ground water, the relative growth rate may decrease and the plant therefore requires less water. The water-use efficiency would however need to be determined to verify this. Furthermore when a branch is cut off, the leaves respond within 10 minutes by causing the pinnules to fold, thereby reducing the surface area exposed to the air. This may be an effort to reduce water loss. This response appears to corroborate the suggestion that *A. mearnsii* is not able to tolerate water stress.

L. conocarpodendron showed the opposite trend. The larger trees have a greater transpiration rate per unit leaf area than the smaller trees. This may be indicative of a drought tolerance strategy. The seedlings and young trees would expend as little water as possible thereby conserving the water held in the soil. This would ensure its survival as long as there is not a severe prolonged drought. Once it has reached the water table it may become a water expender, as appears to be the case with tree no. 5.

The apparent increase in the water-use may also partially be due to the increase in the heartwood. The heartwood would hold more deuterium, as described above, temporarily keeping it out of the water flowing through the sapwood, resulting in a seemingly higher transpiration rate. If, as suggested by Kalma et al. (in press), this only results in an overestimation of 10 % it should not affect the trend significantly. It is, however, unclear if the overestimation remains consistent regardless of the amount of heartwood present.

Implications for water resources

Water is becoming a scarce resource in South Africa due to the increasing pressure of the human population and greater demands to sustain the present-day way of life. At present there is only an annual supply of 1 200 m^3 of water available per capita and water is being used at a rate of 1000 m^3 per capita per year (ch15.htm,1997). To alleviate the crisis a policy has to come into effect to decrease unnecessary water loss. The water catchments therefore need to be managed appropriately. The catchments need to be managed in such a way as to increase the water supply without diminishing its integrity with regard to biodiversity.

It has been shown that on a per unit leaf area basis, *A. mearnsii* transpires significantly more water than *L. conocarpodendron*. If we consider that *A. mearnsii* is more invasive and is able to increase its biomass substantially faster than *L. conocarpodendron*, it stands to reason that *A. mearnsii* would deplete available water resources in catchment areas faster than *L. conocarpodendron* and should therefore be removed from catchment areas.

Conclusion

The deuterium tracer method appears to be suitable for determining transpiration rates in the field. Its accuracy could be improved by taking the systematic losses of deuterium into account. This method can be applied more practically than other contemporary methods such as the heat pulse and porometer methods, and at relatively low cost.

L. conocarpodendron was found to transpire significantly less water than *A. mearnsii*. This has implications for the management of catchment areas to ensure an optimal supply of water resources and still maintain functional diversity.

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