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Variation in water use efficiency and $\delta^{13}\text{C}$ levels in *Eucalyptus grandis* clones

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Abstract

This study aimed to determine whether the $\delta^{13}\text{C}$ levels in the foliage and twigs of four *Eucalyptus grandis* clones were related to their water use efficiency (WUE). This relationship has previously been demonstrated in a number of herbaceous species but not in mature trees. The study involved accurate measurements of tree trunk growth and water use over a period of 4 months, with subsequent isotopic analysis of mature foliage from the north and south side of the canopy, and young leaves from the top of the canopy.

The water use efficiencies were found to vary from 5.97×10^{-3} to $12.3 \times 10^{-3} \text{ m}^3 \text{ m}^{-3}$. Significant differences were observed between clonal-mean water use efficiencies averaged over six sampling periods. The average $\delta^{13}\text{C}$ of the mature and young foliage was found to be significantly correlated with WUE. However, the correlation was weak, suggesting that the relationship between $\delta^{13}\text{C}$ and WUE is more complex in trees than suggested in the literature on crop plants. It is suggested that differences between sample trees in carbon allocation and leaf-to-air vapour pressure deficits may account for the poor correlation between $\delta^{13}\text{C}$ and WUE in the four *E. grandis* clones studied.

Introduction

South Africa is a water-limited country with an average annual rainfall of 560 mm year^{-1} . This results in fierce competition for the limited water resource. In this country, the water use of exotic plantations is a particularly sensitive issue because the areas of most intense afforestation coincide with the country's most important catchment areas. A permit system is in place to regulate afforestation in these areas. Currently, afforestation permits are granted or refused based on an assessment of the expected impact of plantations on water yields from the catchment areas. In future, however, it is likely

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that water will be allocated to competing users on the basis of an assessment of the benefits accrued by the various users.

Water use efficiency (WUE) is a measure that relates biomass production to the consumptive water use of plants. This could provide an objective basis for comparison between alternate agricultural land uses and help resolve issues relating to the allocation of the limited water resource in this country.

As yet no estimate is available of how much water is required for the production of a unit quantity of wood in the South African forestry region. Also, given the concern regarding the hydrological impacts of afforestation, it would be desirable to select trees that use water more efficiently without compromising wood production. Furthermore, a knowledge of WUE would aid in the selection of species for the afforestation of marginal sites, since trees that use water efficiently may produce more timber on sites where water is limiting.

Differences in the WUE of poplar clones were documented by Blake et al. (1984), while Herwitz and Gutterman (1990) indicated similar differences between species of eucalypts planted in the Negev desert. There is thus a possibility that some clones of *Eucalyptus grandis* might be more efficient users of water than others.

The prospect that the ratio of ^{13}C to ^{12}C (typically referred to as $\delta^{13}\text{C}$) in plant tissues could be used to screen for WUE in tree breeding programmes has been derived from demonstrated relationships in crop plants (Farquhar and Richards, 1984; Hubick et al., 1986; Martin and Thorstenson, 1988) and natural communities (Read et al., 1991). The theoretical basis for the link between $\delta^{13}\text{C}$ and WUE is discussed in detail in Francey and Farquhar (1982), Farquhar et al. (1982, 1989), and Farquhar and Richards (1984). The basis of the link is derived, first, from the discrimination between the heavy, ^{13}C , and light, ^{12}C , forms of carbon by the carboxylating enzyme rubisco and, second, from the difference in the diffusivities of water vapour and carbon dioxide in air. In essence, the theory predicts that the least water use efficient plants should have relatively less of the heavier isotope, ^{13}C . Conversely, plants with a high water use efficiency should have relatively more of the heavier isotope, ^{13}C , resulting in a less negative $\delta^{13}\text{C}$.

As yet, the relationship between $\delta^{13}\text{C}$ and WUE has not been demonstrated in trees. Bond and Stock (1990) found differences in $\delta^{13}\text{C}$ levels between eucalypt clones; more negative $\delta^{13}\text{C}$ levels were associated with more productive trees. However, this is the first attempt at linking $\delta^{13}\text{C}$ to WUE in commercial forest trees.

The aim of this project was to estimate the WUE for a range of *E. grandis* clones and determine whether this could be related to the $\delta^{13}\text{C}$ levels in the leaves. The key questions were: (1) how much water is typically transpired

during the production of a unit volume of wood in *E. grandis* during the growing season? (2) does the WUE vary between clones of *E. grandis*? (3) is variation in water use efficiency reflected in the $\delta^{13}\text{C}$ levels in the foliage of *E. grandis* clones?

Materials and methods

Site description and tree selection

Four clones were selected to represent a range from poor to vigorous growth in a clonal *E. grandis* trial at Frankfort State Forest (24°49'S, 30°43'E) in the eastern Transvaal province of South Africa. All four clones are also currently used in commercial plantings in this country. The trial (No. 1/01/08/06/EA62/02) was planted in December 1986 and contains 81 clones from improved, local *E. grandis* stock. Three trees were selected at random from each clone for water use and growth measurements. At the start of the experiment the trees were 4 years old with an average height of 20.4 m.

The trial is situated at 960 m above sea-level, has a mean annual temperature of 18°C and receives an average of 1400 mm rainfall per annum. The soils in the area are typically deep, red, well-drained ferrasols of the Hutton formation (McVicar et al., 1977).

Sampling periods

This study was carried out over 4 months (128 days) beginning on 6 December 1990 and ending on 12 April 1991. This period was divided into six sample periods corresponding to each set of growth measurements. The dates and durations corresponding to each period are presented in Table 1.

Climate

The prevailing weather conditions were recorded at two automatic weather stations. The first was situated in a clearing approximately 500 m from the study site. At this station, photosynthetically active radiation (PAR), relative humidity (*RH*) and temperature were recorded. *RH* and temperature were recorded using a MCS 174 sensor fitted with a Coreci capacitance sensor placed at a height of 1.2 m above ground level. PAR was recorded with a Licor LI190-SB sensor. These data were recorded by a MCS 120 data logger programmed to calculate hourly average PAR, *RH* and temperature. The vapour pressure deficit (VPD) was calculated from the humidity and tempera-

Table 1
The start dates and durations of each of the six sample periods

Period	Start date	Number of days
1	6.12.90	36
2	11.01.91	20
3	31.01.91	12
4	12.02.91	18
5	2.03.91	19
6	21.03.91	23

ture data. Daytime averages of VPD and PAR were calculated using those data for which the hourly average PAR was greater than $5 \mu\text{E m}^{-2} \text{s}^{-1}$.

Rainfall was recorded by a tipping bucket rain gauge connected to a MCS 120 automatic data logger situated at a second weather station approximately 3 km distant from the study site.

Water use determination

The total water use of the selected trees was measured using the 'Custom' Heat-pulse velocity (HPV) recorder and sensor system (Soil Conservation Centre, Palmerston North, New Zealand). The HPV apparatus has been tested on *E. grandis* and shown to provide accurate measurements of water use (Olbrich, 1991).

HPV probe implantation

Prior to probe implantation a survey was conducted to determine the approximate sapwood thickness in each of the four selected clones. As four probes were used for measurement in each tree, the sapwood thickness was used to calculate the depth at which each probe should be implanted so that each probe would sample approximately 25% of the sapwood cross-sectional area.

Four sets of three, vertically aligned holes were drilled radially into the sapwood of each tree at equally spaced positions around the trunk. A 20 mm thick drilling jig was strapped against the stem to ensure that the holes would be drilled parallel to each other. A line heater was inserted into the central hole, while temperature-sensing probes were implanted 10 mm above and 5 mm below the heater. Each temperature probe contained a single thermistor giving a point estimate of sap flow. Four probes were implanted to the desired depth in each of the 12 trees and remained implanted over the period of the study.

HPV logger allocation

Four HPV loggers, each with a capacity to handle four probes, were available for this study. The aim of the study was to accumulate a continuous record of the total water use of each of the 12 selected trees over the 6 month duration of the experiment. Transpiration flow was monitored continuously on one of the 12 trees (the reference tree), and related to the rates recorded on the other 11 trees, sampled on a discontinuous basis. This entailed having one of the four loggers dedicated to the reference tree for the entire duration of the study, while the three remaining loggers were rotated amongst the remaining 11 trees. These three loggers were moved to a different set of trees approximately every 5 days and sap flow (transpiration rate) recorded at half-hourly intervals. In this way it was possible to build up pairwise comparisons between the reference tree and all 11 sample trees.

Ultimately, the relationships between the reference tree and the 11 other sample trees were established using regression techniques. These relationships were then used to estimate the transpiration rates of the 11 sample trees for those periods where data were missing. The complete data set constructed in this way was finally used to estimate the cumulative water use of all 12 trees over the duration of the study.

HPV data analysis

The HPV data were processed using the formulae for the compensation technique as outlined in Swanson (1983). Heat pulse velocities were corrected for wounding using Swanson and Whitfield's (1981) wound correction coefficients.

On completion of the study, all the sample trees were felled and the sapwood area, probe separation, wound size, moisture content of the sapwood and basic sapwood density were measured. These parameters were estimated as described below.

Sapwood area. The final sapwood area of each of the sample trees was determined on a cross-section of the stem containing the HPV probes. Methyl orange stain was used to discriminate between sapwood and heartwood (Bamber and Fukazawa, 1985). The sapwood area was estimated by subtracting the total heartwood area (calculated from the average heartwood diameter) from the total cross-sectional area (calculated from the average under-bark diameter).

The change in sapwood area over the course of the study was estimated by assuming constant sapwood thickness and adjusting the under-bark diameter used in the calculations according to the measurements made at each sample interval.

Probe separation. The probe separation was measured directly off excised sections of the stem containing the probe implantation holes. Measurements were made at the precise depth to which the thermistor was implanted in each set of holes.

Wound size. The wound size was also measured on an excised section of the stem at the site of probe implantation. This was measured for each tree because estimates of transpiration have been shown to be particularly sensitive to errors in this parameter (Olbrich, 1991).

Determination of the moisture content and basic wood density of the sapwood. The procedure outlined by Olbrich (1991) was followed. The average of four sapwood samples was used in the data analyses.

Tree growth

The growth increment of each of the sample trees was measured approximately every 3 weeks. This was necessary to calculate the volume increase of each of the sample trees over the course of the study.

Stem girth

The stem girth was measured at four positions on the stem: at breast height, 4, 8 and 11 m above the ground. Measurements were made using two systems. First, expandable aluminium collars were used to document the diameter increments of the sample trees at the four heights. This involved making an accurate mark on the metal band adjacent to a reference point. The diameter increment was then calculated by measuring the distance between successive marks on the band. Second, a diameter tape placed immediately above the diameter collars was used to measure stem diameter directly. Measurements were always made before 10:00 h in the morning to avoid the possible inaccuracies associated with stem shrinkage at high transpiration rates.

Tree heights

Simultaneous measurements were also made of the total tree height. The most successful technique was to use extendable height rods placed on top of a 10 m ladder strapped against the tree. An observer standing some distance away from the base of the tree would then judge whether the top of the height rod corresponded to the top of the tree.

The accurate measurement of tree height posed some problems because it was often difficult to determine the exact position of the tip of the height rod in relation to the top of the tree. In addition, a wind storm during March resulted

in many of the tree tops bending over. This resulted in some measurement error as it became necessary to estimate tree height as if the tip had not bent over. The tips did straighten out after several weeks, allowing precise measurement to resume. Where errors resulted in an apparent decrease in tree height, the height was estimated graphically assuming an approximately linear increase in height over the period for which the data was erroneous.

Tree volume

The shape of the four lower sections of the stem was assumed to be that of a frustum of a cone, while the shape of the apical section was assumed to be conical. The stem volume was calculated by summing the volumes of the five stem sections, with volume increment calculated by subtracting the final volume from the initial volume.

On completion of the study the leaves were stripped off all the sample trees, weighed and leaf areas calculated using a fresh mass:leaf area relationship previously determined for *E. grandis* in this area.

Determination of WUE

The WUE was calculated for each sampling interval by dividing the increase in stem volume by the cumulative transpiration rate recorded for that period. The WUE data are presented in three ways: first, WUE was calculated as described above, second, the average WUE of the six sampling periods, denoted WUE_{av} , and third, season-long WUE which was calculated by dividing the total growth over the study period by the total water use, denoted WUE_{sl} .

It should be stressed that the usage of the term water use efficiency is not based on an estimate of whole-plant production, but, specifically on stem-wood production. This is in line with other studies such as that performed by Kaufmann (1985), and reflects the difficulty of collecting quantitative data on below-ground carbon allocation in deep-rooted trees.

Foliage sampling for stable carbon isotope analyses

Mature leaves from north and south aspects, and young leaves from the top of the tree canopies, were sampled immediately after the trees were felled. Young leaves were sampled from the growing tips of the uppermost branches of the canopy. Mature foliage was sampled from older branches midway in the canopy. Approximately 100 leaves were collected from each of the three

canopy positions. After collection, samples were dried at 70°C and finely ground to a 60 mesh size in a Wiley mill.

Plant material was combusted in sealed quartz tubes according to the method described by Sofer (1980). Carbon dioxide formed in the tubes was collected by cryogenic distillation on a vacuum line into pyrex tubes (Sealy, 1986). The $\delta^{13}\text{C}$ values of the samples were determined on a VG Micromass 602E mass spectrometer using a reference gas calibrated against six National Bureau of Standards isotopic reference materials, NBS 16–21. All isotope ratios are expressed using the Pee Dee Belemnite Standard, where $\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$. A sample reproducibility of 0.2‰ was determined.

Results

Climate

The climate during the study period was fairly typical for the region except for the relatively dry weather experienced during April. Most of the rain fell during the early stages of the study with the highest mean daily rainfall

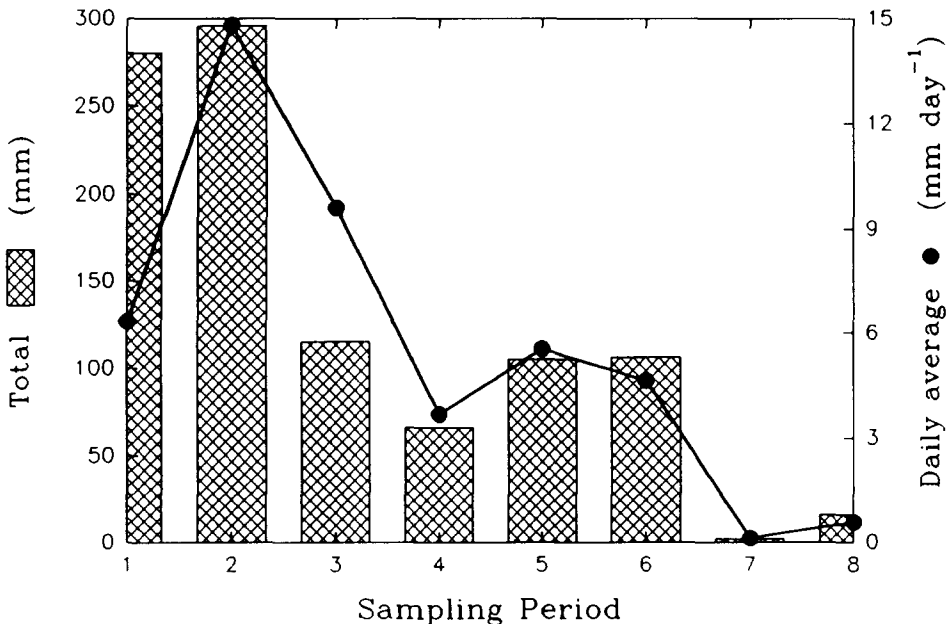


Fig. 1. The total (bars) and average daily (solid line) rainfall during each sample period.

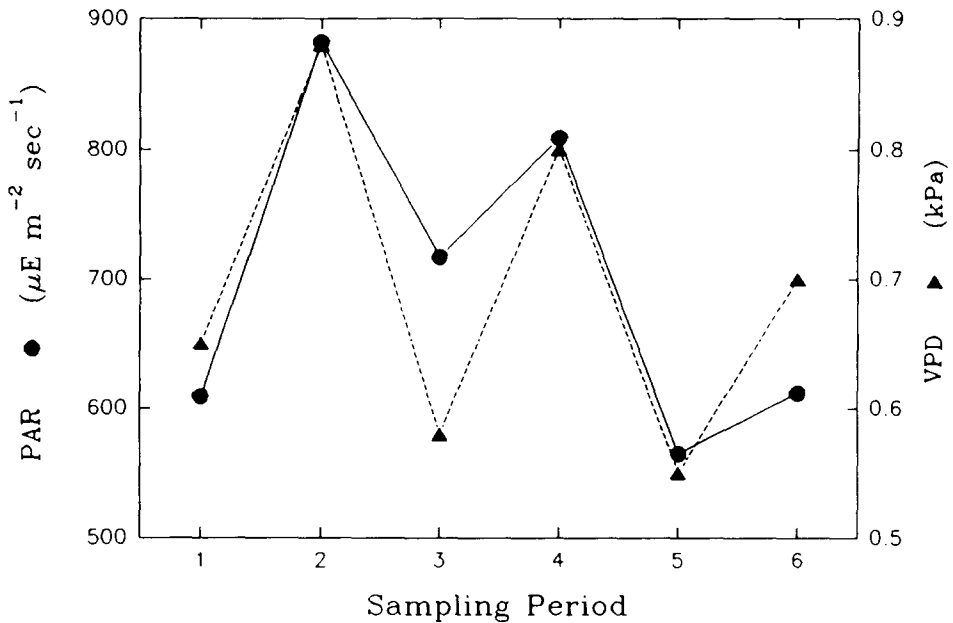


Fig. 2. The daily average daytime photosynthetically active radiation (PAR), and vapour pressure deficit (VPD) during each of the six sample periods.

occurring during period 2 (Fig. 1). The total rainfall during the study was 984 mm, marginally above the 30 year mean for that 4 month interval.

Transpiration from *E. grandis* in the eastern Transvaal has been shown to be mainly a function of the vapour pressure deficit (VPD) and the level of photosynthetically active radiation (PAR) (Dye and Olbrich, 1993). The mean daytime levels of VPD and PAR fluctuated over the course of the study (Fig. 2). The highest VPD were recorded during periods 2 and 7 with mean

Table 2

Mean (\pm SD) height, diameter at 1.3 m, leaf area and sapwood area of the three sample trees for each of the four clones, determined at the end of the study

Clone	Height (m)	Diameter at 1.3 m (cm)	Leaf area (m ²)	Leaf-area: sapwood-area ratio (m ² cm ⁻²)
A	22.90 \pm 0.51	14.0 \pm 1.0	23.8 \pm 4.1	0.34 \pm 0.04
B	23.38 \pm 1.39	17.5 \pm 3.0	40.9 \pm 17.7	0.32 \pm 0.03
C	23.51 \pm 0.59	17.2 \pm 1.5	37.1 \pm 10.3	0.31 \pm 0.06
D	23.14 \pm 2.03	15.4 \pm 3.0	31.4 \pm 4.5	0.21 \pm 0.04

Table 3

The mean clonal increases in the height, diameter and volume of the sample trees over the duration of the experiment

Clone	Height (m)	Percentage change in stem diameter at four heights				Stem volume (m ³)
		1.3 m	4 m	8 m	11 m	
A	1.1 b	5.4 bc	10.0 ab	17.8 a	50.7 a	0.0276 a
B	1.9 a	11.6 a	13.0 a	20.3 a	41.2 ab	0.0531 a
C	1.1 b	9.2 ab	11.5 a	20.1 a	30.8 b	0.0425 a
D	1.7 ab	4.3 c	6.6 b	11.9 b	36.2 b	0.0268 a

Means were tested for significance using Duncan's multiple range test and means with the same letter are not significantly different at $\alpha = 0.05$.

daytime values of approximately 0.85 kPa. The general decline in PAR over the study reflects the lower azimuth during the winter months.

Growth

At the end of the study all the trees were between 21 and 25 m tall and had a diameter at 1.3 m of between 12.5 and 20 cm (Table 2). This reflects an average of approximately 5 m height-growth per year over the 4 years, illustrating the growth potential of this species in this area. Clones A, B and C had similar leaf area to sapwood area ratios, while clone D had considerably less leaf area per unit sapwood area (Table 2).

Table 3 illustrates the relative growth of the four clones studied. Clone B showed the greatest mean increase in stem volume over the course of the study. Clone C was second due to its superior increase in stem diameter, followed by clones A and D. The stem volume increase measured in clone B was almost double that of clones A and D.

The percentage increase in stem diameter was greatest in the measurements made immediately beneath the canopy (at 11 m above the ground), the diameter of clone A's stem increasing by more than 50% over 128 days (Table 3). Although the growth increments were found to be well correlated at all the levels within a single tree, the most sensitive point for growth measurements was immediately beneath the canopy. It is these dramatic increases in tree size over such a short time that make this species suitable for a WUE study of this nature.

All trees studied showed the same basic trend in growth during the study, with a peak during the second period (January/February) and only minor fluctuations over the remainder of the study (Fig. 3). Despite the similar

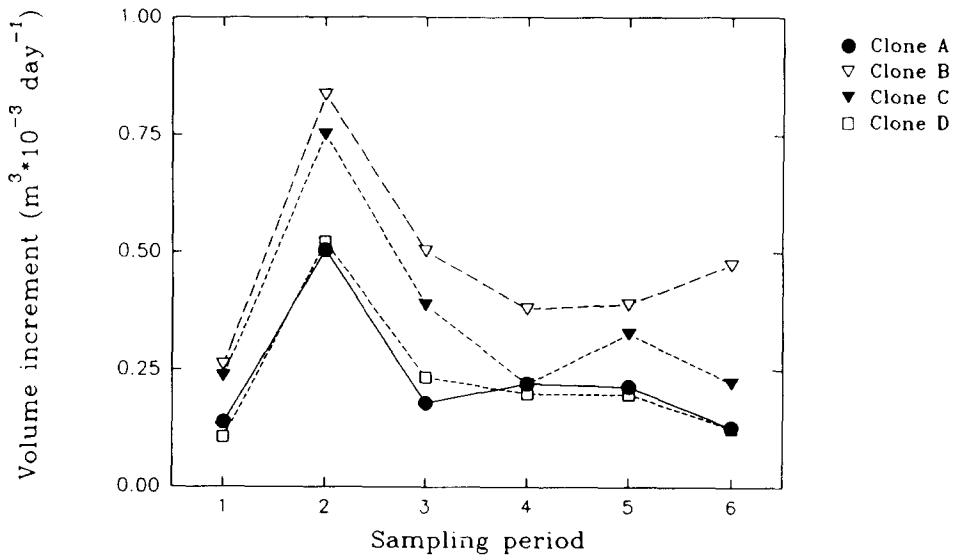


Fig. 3. The clonal-average daily volume increments measured in $m^3 \text{ day}^{-1}$.

pattern in growth, there was considerable variation in the actual daily volume increment both between individuals within a clone and between clones.

It was surprising to find that the growth during the first period (December/January) was often lower than that recorded during period 2. It would appear

Table 4
Regressions estimating total daily transpiration ($l \text{ tree}^{-1} \text{ day}^{-1}$) for each of 11 sample trees

Tree	Clone	Regression	No. days sampled	R^2
1	A	$y = 0.966x + 0.983$	18	0.98
2	A	$y = 0.670x + 0.315$	32	0.96
4	B	$y = 0.657x + 0.0594$	7	0.99
5	B	$y = 1.31x + 3.83$	18	0.96
6	B	$y = 2.72x + 5.31$	56	0.90
7	C	$y = 1.28x + 2.33$	11	0.98
8	C	$y = 1.30x + 3.88$	56	0.95
9	C	$y = 1.15x + 1.91$	9	0.99
10	D	$y = 1.78x + 3.14$	11	0.99
11	D	$y = 1.05x + 3.18$	55	0.98
12	D	$y = 0.659x + 2.36$	18	0.96

Regressions were established by correlating daily transpiration from the anchor tree (x) with sample data taken at intervals from each of the remaining 11 trees (y)

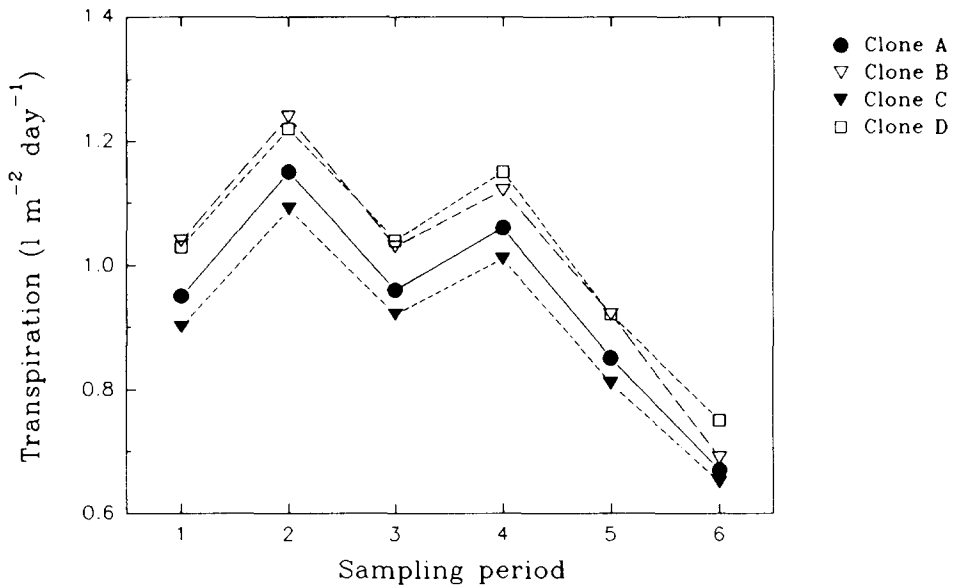


Fig. 4. The clonal-average daily water use per square metre of foliage.

that growth is regulated quite strongly by climatic variation and not by season alone. The levels of PAR and VPD during the first period were particularly low (Fig. 2), implying that the low temperatures and limited radiation resulted in reduced growth rates. Conversely, period 2 had much higher radiation and temperature levels (Fig. 2) which is perhaps what stimulated the growth peak. Growth also appeared to be well correlated with daily average rainfall.

Water use

In all cases the daily total water use of the reference tree and the other sample trees was linearly related with a high correlation ($R^2 > 0.90$, Table 4). This permitted the precise calculation of total water use in the remaining 11 sample trees using the relationships described in Table 4. The regressions were characterized by large variation in the x -coefficients (0.66–2.7). This reflects differences in the total leaf area of the selected sample trees, trees with larger canopies having higher absolute transpiration rates.

The average daily transpiration varied over the course of the study (Fig. 4). Peak transpiration rates coincided with peaks in the average daytime PAR and VPD recorded during period 2 (Fig. 2). Transpiration was standardized by expressing it on a leaf area basis, this resulted in a tight grouping in the four curves (Fig. 4). This figure shows that, per unit leaf area, clone C was the most conservative water user, clones B and D were more liberal and clone A was

Table 5

A summary of the clonal means (\pm SD) of: growth, water use, water use efficiency (WUE_{av} and WUE_{sl}), and $\delta^{13}\text{C}$ levels in the leaves

Attribute	Clone			
	A	B	C	D
Growth (m ³)	0.028 \pm 0.0006 a	0.053 \pm 0.022 a	0.043 \pm 0.009 a	0.027 \pm 0.014 a
Water use (m ³)	2.58 \pm 0.53 a	4.76 \pm 3.18 a	3.90 \pm 0.33 a	3.68 \pm 1.68 a
WUE _{av} (m ³ m ⁻³ \times 10 ⁻³)	9.44 \pm 0.93 a b	12.3 \pm 3.88 a	9.50 \pm 2.56 a b	5.97 \pm 0.73 b
WUE _{sl} (m ³ m ⁻³ \times 10 ⁻³)	10.8 \pm 0.850 a	13.2 \pm 4.28 a	11.1 \pm 2.61 a	7.13 \pm 0.411 a
$\delta^{13}\text{C}$ (‰)	-23.3 \pm 0.17 a	-28.0 \pm 0.08 a	-28.3 \pm 0.05 a	-28.8 \pm 0.34 b

Means were tested for significance using Duncan’s multiple range test and means with the same letter are not significantly different at $\alpha = 0.05$.

intermediate. Average daily water use declined for all trees towards the end of the study as autumn approached.

Water use efficiency

The season-long water use efficiencies (WUE_{sl}) were consistently higher than the average over the six measurement periods (Table 5). The total water use of the individual trees varied from 1.96 to 8.18 m³ while growth varied from 1.43 \times 10⁻² to 7.14 \times 10⁻² m³ over the study period. The clonal-average WUE_{sl} varied from 0.066 to 0.017 m³ wood produced per m³ water consumed. The significance of differences in the WUE between clones depended on the way in which WUE was calculated. No significant differences were found between clones in terms of WUE_{sl} (Table 5), but clone D was found to be significantly lower than clone B in terms of WUE_{av}.

Three of the four clones had an average WUE_{sl} of between 0.011 and 0.013 m³ m⁻³. The fourth clone, clone D, had the lowest water use efficiency of 0.007 m³ m⁻³. Clone D, described as a liberal water user (Fig. 4), had one of the slowest growth rates (Fig. 3), and had a sapwood-area to leaf-area ratio which differed markedly from the other three clones (Table 2).

The WUE was also found to vary over the duration of the study (Fig. 5). In general, all the trees followed the same basic trend, although differences in WUE between clones were more marked than was the case with growth

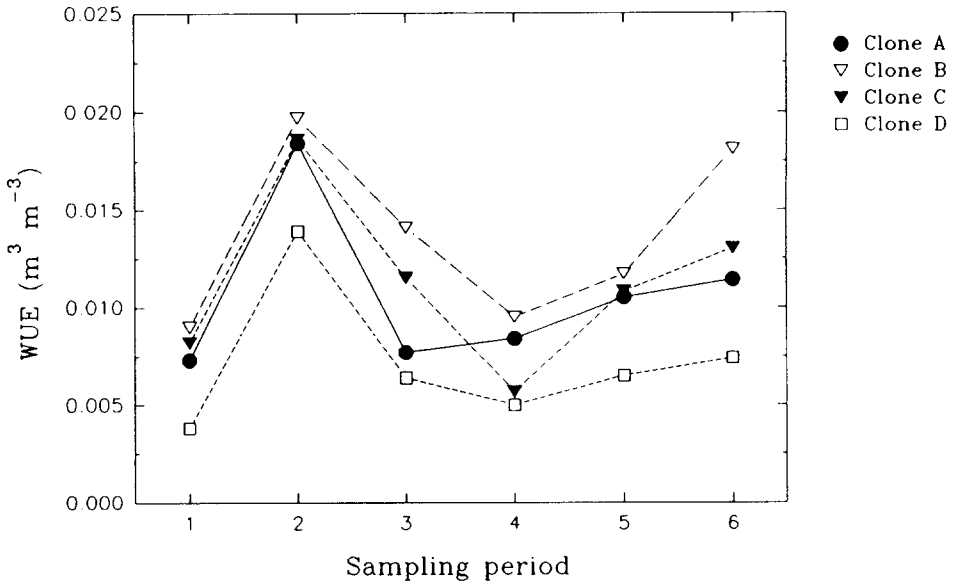


Fig. 5. The clonal-average water use efficiency in m^3 stem volume increase per m^3 water transpired, recorded for each of the six sampling periods.

increments (Fig. 3). Two trends were evident: there was a peak in WUE corresponding to the peak in growth during period 2, and there appeared to be a general increase in WUE as the dry winter period approached (Fig. 5). This seems to indicate two factors which influence water use efficiency. First, during the rainy season conservation of water is not a priority, and high WUE is, therefore, only achieved during periods of rapid growth. Second, once the dry season approaches, the conservation of water becomes more important and the plant exercises a tighter control on transpiration, but still produces stem wood at a relatively high rate, resulting in a higher WUE.

The WUE_{av} was particularly variable in clones B and C (Table 5, Fig. 6). The large variation in WUE_{av} recorded in clone B was primarily due to the low WUE_{av} of tree 6. Two of clone B's sample trees had a relatively high water use efficiency, while the third (tree 6) was considerably lower, resulting in the large estimate of interclonal variation.

The relationship between $\delta^{13}\text{C}$ and water use efficiency

The $\delta^{13}\text{C}$ values of the leaves sampled varied between -25.22 and -29.66‰ , which is typical for C_3 plants (O'Leary, 1981). Significant clonal differences were found in the average $\delta^{13}\text{C}$ of the leaves sampled from the canopy (Table

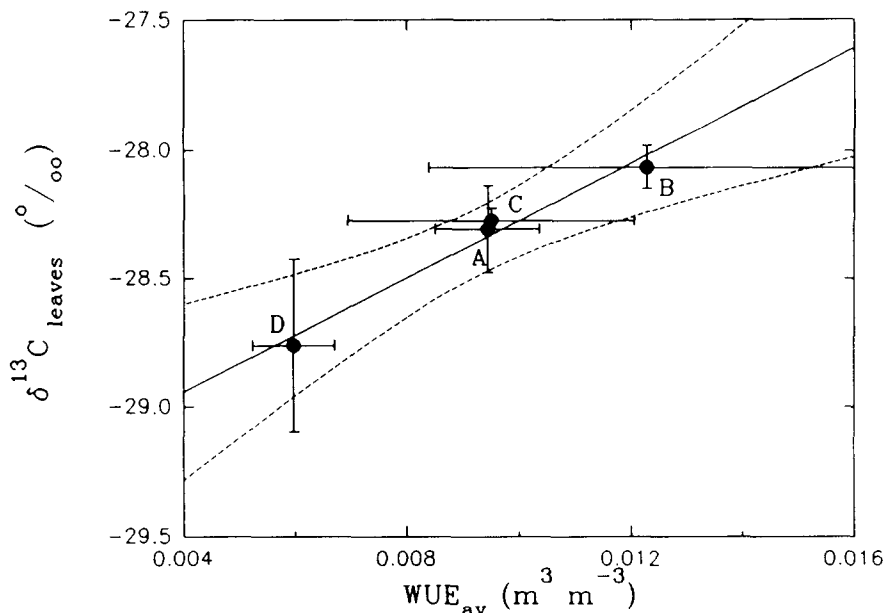


Fig. 6. The relationship between the average water use efficiency (WUE_{av}) of the four clones and the mean $\delta^{13}C$ level of leaves from the three canopy positions sampled (mature leaves from the northern and southern side of the canopy, and young foliage). The error bars represent 1 standard deviation of the mean and the lines represent the regression between clonal-mean WUE_{av} and $\delta^{13}C$ with the associated 0.95 error limits ($R^2 = 0.44$, $p = 0.012$).

6). The clone with the lowest WUE, clone D, was found to have significantly more negative $\delta^{13}C$ values than the other three clones.

The relationship between $\delta^{13}C$ and WUE again depended on the way in which WUE was calculated. $\delta^{13}C$ was not significantly correlated with WUE_{sl} for any of the tissues sampled. However, significant correlations were observed between WUE_{av} and the average $\delta^{13}C$ level recorded for the leaves ($p = 0.012$, adjusted $R^2 = 0.44$).

The difference in WUE_{sl} and WUE_{av} is that the first measure integrates

Table 6
Clonal mean $\delta^{13}C$ values of the leaf tissue sampled from the 12 *E. grandis* sample trees

Clone	North	South	Young	Average
A	-28.76 a	-29.27 a	-26.50 a	-28.31 a
B	-28.56 a	-28.78 a	-26.86 a	-28.07 a
C	-28.85 a	-28.78 a	-27.77 a	-28.27 a
D	-28.97 a	-29.03 a	-27.44 a	-28.76 b

Means were compared for significance using Duncan's multiple range test, means with the same letter are not significant at $\alpha = 0.05$.

water use and growth over the course of the study, while the second is an average weighted by the number of periods with relatively low WUE (Fig. 5). The foliar samples were collected at the end of the study which followed a period characterized by generally low WUE. This may explain why $\delta^{13}\text{C}$ was only correlated with WUE_{av} .

The relationship between $\delta^{13}\text{C}$ and WUE may be obscured by variation caused by microclimatic differences in the canopy and varying proportions of organic compounds bearing different isotopic signatures (unpublished data). This may explain why only the average $\delta^{13}\text{C}$ of several samples was correlated with WUE_{av} .

Discussion

It should be stressed that the usage of the term water use efficiency is not based on an estimate of whole-plant production, but specifically on stem-wood production. This is in line with other studies such as that performed by Kaufmann (1985), and reflects both the difficulty of obtaining quantitative data on below-ground carbon in trees and also the perspective of the commercial forester.

The interclonal differences between season-long growth and water use were not significant, despite the means differing by up to 100% (Table 5). Differences in the clonal-mean water use efficiencies (WUE_{sl}) were also dramatic, resulting in 86% more stem-wood production in the most efficient clone, clone B, per unit volume water consumed than the least efficient clone, clone D (Table 5). A general trend was evident which suggests that WUE was related to growth rate, with the most water use efficient clones tending to be the fastest growing. For example, despite the fact that clone B used the most water, it was still the most efficient user of water because it also grew the fastest.

Honeysett and Beadle (1987) recorded water use efficiencies of $0.0112 \text{ m}^3 \text{ m}^{-3}$ for *E. delegatensis* and $0.0188 \text{ m}^3 \text{ m}^{-3}$ for *E. nitens* trees of approximately 3.5 years old. These values are similar to those found in this study. They comment that although the water use levels of the two species did not differ dramatically, there were substantial differences in growth rates. This suggests that the observed differences in WUE were largely due to differences in stem growth rate.

There was considerable variation in both WUE_{av} and the $\delta^{13}\text{C}$ level in the leaves (Table 5), even within clones. Intuitively, less variation was expected in these two parameters within clones because the trees studied grew on the same site, often within a few metres of one another, and were genetically identical. R.H. Waring and W.B. Silvester (personal communication, 1993) confirm that large variation in $\delta^{13}\text{C}$ is commonly found within individual plants, especially

trees. They found that aspect and branch length accounted for a 6‰ $\delta^{13}\text{C}$ difference within a canopy. It is still unclear how $\delta^{13}\text{C}$ varies within the canopy of eucalypts, and how sensitive the estimate is to differences in sampling. It may be crucial to follow a rigid sampling procedure, where a specific tissue-type is sampled at a precise location within the canopy.

Clonal $\delta^{13}\text{C}$ values derived in this study showed a potential link between $\delta^{13}\text{C}$ and WUE in 4-year-old *Eucalyptus* trees. The only significant differences to and significant correlations with WUE were found in the average $\delta^{13}\text{C}$ level recorded in foliage sampled from three positions in the canopy. This suggests that the relationship between WUE and $\delta^{13}\text{C}$ in trees is not as simple as was demonstrated in tomato, wheat, and peanut genotypes (Farquhar and Richards, 1984; Hubick et al., 1986; Martin and Thorstenson, 1988).

The poor correlations observed between $\delta^{13}\text{C}$ and WUE_{av} may, in part, be due to differences between trees in the canopy microclimate. A comparison of $\delta^{13}\text{C}$ values to indicate differences in WUE between trees is only valid with equal VPD (Farquhar et al., 1989). However, there was considerable variation in the observed transpiration rates of trees within clones (Table 5) and it is likely that these differences resulted in differences in the leaf-to-air VPD experienced by the various canopies. Differences in transpiration rates are also likely to result in differences in leaf temperature. Ehleringer et al. (1992) showed that this factor also influences $\delta^{13}\text{C}$ values, further confounding the comparison of the relationship of $\delta^{13}\text{C}$ to WUE in the present study.

The figures illustrating stem volume increment (Fig. 3) and water use (Fig. 4) show that stem growth and water use were poorly coupled over the course of the study. Given that the processes of carbon uptake and transpiration are linked (at the leaf level), this suggests that results of short-term studies, such as this one, may be confounded because the allocation of carbon to stem growth does not reflect total carbon uptake.

This study found that the season-long water use efficiencies of the four *E. grandis* clones studied varied between $0.013 \text{ m}^3 \text{ m}^{-3}$ and $0.007 \text{ m}^3 \text{ m}^{-3}$ during the latter half of the summer. Only the differences between clones at the extremes of WUE range were significant. Also, the use of stable carbon isotopes to screen for WUE still holds promise. However, there is still a need to refine our understanding of the relationship between $\delta^{13}\text{C}$ and WUE in *Eucalyptus* trees by identifying and quantifying the sources of the variation observed that may obscure the direct link between the two parameters.

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