

**THE APPLICATION OF THE  
HEAT PULSE VELOCITY TECHNIQUE  
TO THE STUDY OF TRANSPIRATION  
FROM Eucalyptus grandis.**

**Bernard W. Olbrich**

## PREFACE

All research described in this thesis was carried out in the Department of Biology, University of Natal, Durban from February 1989 to July 1993 under the supervision of Professor N. Pammenter.

The studies represent original work by the author and have not been submitted in part or in whole to this or any other University.



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Bernard Wolfgang Olbrich

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Date

## ABSTRACT

This thesis examines the application of the heat pulse velocity technique (HPV) to plantation-grown *Eucalyptus grandis* in the Eastern Transvaal, South Africa. The work addresses the application of the technique *per se* and is ultimately focused on improving the prediction of the hydrological impact of afforestation, to assist in the equitable management of South Africa's limited water resources.

The verification of the HPV technique on *E. grandis* against the cut-tree method showed that the technique accurately reflected the water uptake in four three-year-old trees and a sixteen-year-old tree. It was found that accurate measurement of wound size and probe separation was essential for accurate water use estimates.

The optimal probe allocation strategy for accurate measurements of transpiration in individual trees and stands of trees was examined. Stratifying the depths of implanted probes resulted in greater precision and repeatability in the HPV-derived estimates of sap flow in *E. grandis*. Given a limitation in the number of probes available to estimate stand transpiration, the results showed that sampling many individuals with a low sampling intensity (few probes per tree), rather than sampling few individuals intensively, improved the estimate of stand transpiration.

An examination of the influence of tree age and season on transpiration rates showed that the transpiration rate per unit leaf area of *E. grandis* declined with age. Also, transpiration rates were higher in summer than under equivalent conditions of evaporative demand in winter. A seasonal change in the response of transpiration to VPD was implicated as the primary cause of this shift. A number of models were

derived to predict transpiration from *E. grandis*. The variables vapour pressure deficit (VPD) and photosynthetically active radiation (PAR) were found to account for a large proportion of the observed variation in transpiration from the age sequence of trees studied. The models developed are applicable to trees of varying age, but are valid only for conditions where minimal soil water stress is experienced.

The derived models were tested against two sets of independent data. This confirmed that a simple linear multiple regression adequately describes the relationship between transpiration and the two driving meteorological variables, PAR and VPD, in *E. grandis*. The application of a selection of the developed models on a sample data set from Sabie showed that transpiration from a three-year-old stand of *E. grandis* in summer may be more than double that for a sixteen-year-old stand under the same conditions. Simulated results also showed that transpiration in summer was about 25 to 50% higher than that from the same stand during winter conditions. Simulated transpiration rates from the young *E. grandis* stands were high, suggesting that further validation of the estimated rates is required before the models are applied.

It is concluded that the HPV method is an ideal technique to estimate water use in *E. grandis* trees. The models developed represent a major advancement on previous models used to predict the hydrological impact of afforestation on mountain catchments.

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Much assistance was given to me during the verification and field application of this technique. Many leaf-strippers were involved in stripping all the leaves off the more than fifty trees sampled in this study. The work of our technical staff, particularly Sam Soko, David Mapanga and Tony Poulter is also acknowledged. Thanks also to Dr. Peter Dye and other hydrology colleagues, in particular Jan Bosch, for their interest and support. Winston Groenewald's assistance in writing the SAS code for the Monte Carlo simulations was also valued.

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# CHAPTER 1.

## GENERAL INTRODUCTION

### 1.1 INTRODUCTION

There is an ever increasing demand for South Africa's limited water resources, due to population growth and industrial development. The number and diversity of user groups competing for water complicates the management and allocation of water resources. Forestry is a major water user, and as such competes with other user groups, such as agriculture. Yet forestry sustains the valuable timber, pulp and associated industries. The Department of Water Affairs and Forestry is also one of the primary agencies charged with the responsibility of the conservation of mountain catchments. In view of its significant contribution to the national economy, and the fact that it does not incur direct water supply costs, forestry has a legitimate claim to water in competition with other users, particularly agriculture, in climatic regions where forestry is viable (Department of Water Affairs, 1986).

Long term rainfall records over the summer rainfall region of South Africa show a quasi-cyclicity, in which approximately nine years of above average rainfall are followed by approximately nine years of below average rainfall (Tyson, 1986). Historically, the conflict between forestry and other competing water users has heightened towards the end of the dry phases in the rainfall cycle. The dry phase ending in 1932 resulted in laws being introduced to protect riparian zones. At the end of the next dry cycle a committee was appointed to investigate problems relating to the conservation of the principle mountain catchments. The dramatic drought in the mid-1960's raised concern that afforestation was causing a serious decline in the water yield from the mountain

catchments. This was confirmed by early South African catchment research experiments. These showed that the afforestation of the winter-dormant montane grasslands with fast-growing, deep-rooted evergreen trees decreased streamflow significantly after only a few years (Nanni, 1956; van Lill *et al.*, 1980, Bosch, 1979). This culminated in an Act, introduced in 1972 by the promulgation of the Forest Amendment Act (Act 46 of 1972), which regulated new afforestation through a permit system. Currently, permits are issued or withheld based on an assessment of the predicted effect of afforestation on runoff, taking into account the existing and expected demands for water within the catchment.

This assessment uses the predictive curves developed by Nanni (1956), from work done on pine plantations in a gauged-catchment experiment in the Natal Drakensberg. However, the applicability of these classic catchment experiments is limited as their results cannot be extended to other forested catchments with different geologies and climates with any degree of confidence (Newson and Calder, 1989). Recognition of this provided the impetus for a process-based understanding of evapotranspiration from South African forest plantations. The aim was to develop models which would ultimately replace the Nanni curves as predictors of the hydrological impact of afforestation. These models should be driven by hourly or daily weather data available from a network of weather stations, and so allow plantation water use to be simulated over a wide range of sites.

The process studies first focused on *Eucalyptus grandis*, because plantations of this species caused a more rapid decline in water yields than pine plantations (van Lill *et al.*, 1980). Also, *E. grandis* is planted over an area in excess of half a million hectares, and accounts for a significant proportion of new afforestation in South Africa (Promotion Committee, 1992).

Dye (1987) initiated an intensive study of stomatal conductance ( $g_s$ ) within a single *E. grandis* tree, and its correlation to environmental variables. He developed a model of canopy conductance by incorporating data on the leaf area distribution in the canopy. The most important environmental variables regulating transpiration were vapour

pressure deficit (VPD) and the level of photosynthetically active radiation (PAR). Soil water availability was not limiting, even at the end of the dry winter season. Also, there was a distinct seasonal shift in the relationship between canopy conductance and the driving environmental variables.

The technique of porometry was used to measure stomatal conductance in the above study. In order to reach the foliage of the study tree, it was necessary to construct a 25 m tall scaffolding tower. As only a single six-year-old tree was used, a question is raised as to the general applicability of the model to a) the stand as a whole, and b) to different-aged stands under similar site conditions. These limitations are common to many studies involving the extrapolation of leaf-based measurements to the whole tree, or to an entire canopy. This thesis examines the use of an alternate technique, the heat pulse velocity technique (HPV), which directly estimates whole-tree water use. The aim of the work described in this thesis is to contribute to the development of process based models to predict the hydrological impact of afforestation, to ultimately replace the predictive curves currently used in the forest permit system. Specifically, the objectives of the thesis are:

- i) To verify that the HPV technique can be used to obtain accurate estimates of the water use of *Eucalyptus grandis*.
- ii) To establish the optimal probe allocation strategy for the measurement of transpiration in *E. grandis*.
- iii) To assess the influence of tree age and season on transpiration from *E. grandis* where soil water is not limiting, and contrast these results to those of Dye (1987).
- iv) To develop a model to predict plantation water use of *E. grandis* growing under conditions of non-limiting soil water availability.

The thesis is presented in six chapters. Chapter two details a series of verification experiments conducted to test the HPV apparatus on *E. grandis*. Chapter three presents the results of the field application of the HPV technique, which examined the influence of season and tree age on canopy transpiration rates. Chapter four



discusses the application of models developed in the age sequence study to a sample data set. Chapter five examines the influence of different probe allocation strategies on the accuracy of the estimate of canopy transpiration. Chapter six presents a general discussion of the major results, and outlines potential limitations and additional research required.

Sections of the thesis have already been published and copies of these papers have been included in Appendix I.

## CHAPTER 2.

# THE VERIFICATION OF THE HEAT PULSE VELOCITY TECHNIQUE FOR ESTIMATING SAP FLOW IN EUCALYPTUS GRANDIS

### 2.1 INTRODUCTION

It is still difficult to measure forest transpiration, despite recent advances in instrumentation and progress with micro-meteorological approaches. Most techniques require a structure either to gain access to the canopy, or to serve as a platform for micro-meteorological instrumentation mounted in and above the canopy. One of the aims of this thesis was to assess the the influence of tree age and season on transpiration from *E. grandis* trees where soil water was not limiting. Given that *E. grandis* trees grow extremely fast and tall in the Eastern Transvaal, this would involve the construction of towers in each stand to be studied, so equipment could be placed above the canopies of the trees. In a stand with trees only 16-years-old this would require a tower in excess of 60 m in height! Studying an age-sequence of trees using such micro-meterological and energy balance approaches would therefore be difficult.

More importantly, energy balance approaches have theoretical requirements for fetch that could not be met in the Sabie area due, firstly, to topographic complexities. Secondly, commercial plantations consist of a patchwork of compartments differing in age and species, intermingled with unplanted areas (bare soil). As a result, the flow of bulk air across these landscapes is complex and turbulent. The unplanted areas would also confound transpiration estimates by introducing a convected energy

component that could not be easily resolved. As a result, the application of a technique such as the Bowen Ratio technique would be untenable.

Eucalypt plantations in the Eastern Transvaal often have very little understorey vegetation, and a relatively high leaf area index in the *E. grandis* canopy. As a consequence, the relative contribution of the understorey vegetation and the soil surface to the evaporative loss is likely to be small compared to the transpiration from the tree canopy. Estimating transpiration from the trees is therefore a priority, and highlights the need for a rapid, simple method for measuring tree transpiration in forest hydrology (Swanson and Whitfield, 1981).

Since all water transpired from the canopy must pass through the stem, this is the logical site for measurement. Estimates of the quantity of water passing up the stem can be made using the heat pulse velocity (HPV) technique (Swanson, 1983). This method is rapid, amenable to automatic data capture, and the instrumentation is portable, allowing estimates on a number of trees during the course of a day.

The concept of using heat as a tracer in the sap stream of trees to measure transpiration is not new (Huber and Schmidt, 1937). Until recently, however, it was not possible to apply the HPV technique to trees without prior calibration (Cohen, Fuchs and Green, 1981; Marshall, 1958; Swanson, 1983). Implanting probes in the stem, and so interrupting sap flow, violates the assumptions of idealized heat transport (Swanson and Whitfield, 1981). It becomes necessary to correct for this by measuring the width of the zone where flow has been interrupted (i.e the wound size). Accurate estimates of transpiration have been attained for trees including *Nothofagus solandri*, *Pinus halepensis*, *P. radiata* (Swanson and Whitfield, 1981), and *Malus sylvestris* (Green and Clothier, 1988), using the HPV technique with a correction factor for the wound size, which is measured on completion of the experiment. There remains some doubt as to how well the technique actually works for some species, for example Hatton and Vertessy (1990) report that the heat pulse method consistently exceeded estimates of evaporation based on bowen ratio measurements in *Pinus radiata*.

It was therefore necessary to establish how accurately the heat pulse technique reflected actual sap flow in *E. grandis* and refine the application of the technique for the species. This chapter specifically outlines the verification and application of the HPV technique to a diffuse porous hardwood species, *Eucalyptus grandis*, the major plantation-grown hardwood in South Africa.

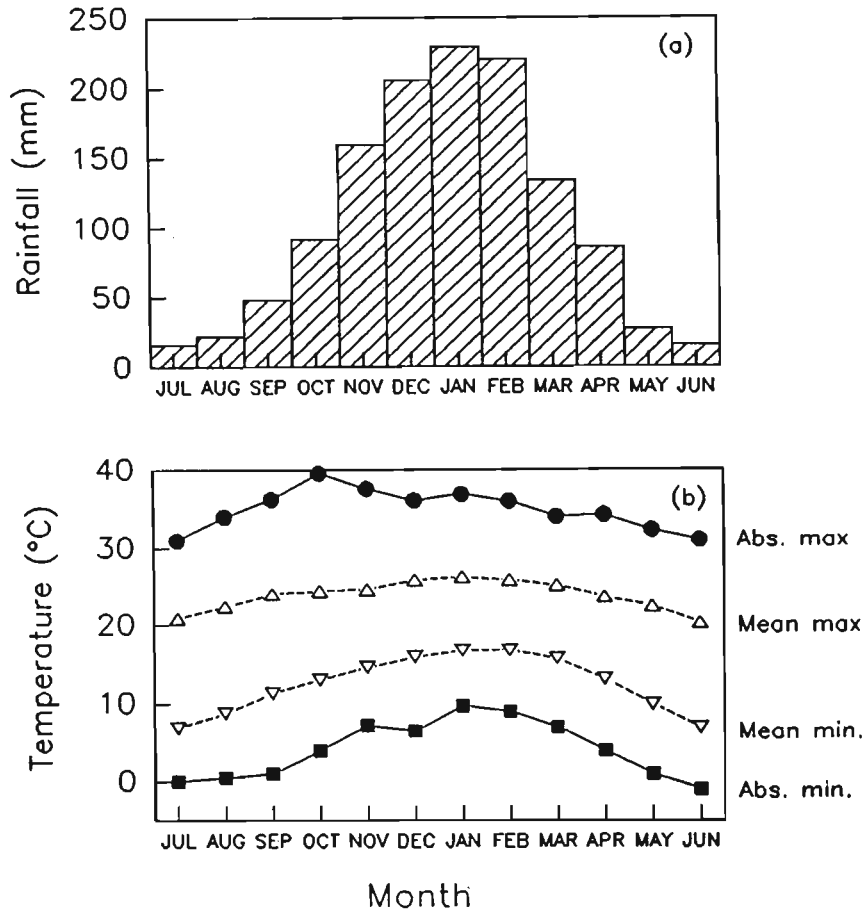
The specific objectives of this study were:

- (i) to investigate how accurately the HPV technique could measure the amount of water flowing up the stems of two age classes of *E. grandis* trees, and
- (ii) to determine the sensitivity to errors in the measurement of the various parameters required to calculate sap flow.

## **2.2 MATERIALS AND METHODS**

### **2.2.1 Site description**

Trees were selected from stands of plantation-grown *E. grandis* on Frankfort State Forest in the Eastern Transvaal, South Africa (25°03'S and 30°53'E). In this area *E. grandis* trees are fast-growing and growth rates in excess of 5 m per year are not uncommon. The area is situated 1100 m above sea level, has a mean annual temperature of 18.1°C, and receives an average of 1230 mm rainfall per annum falling predominantly in the summer months (Figure 1a). Temperatures below 0°C are extremely rare, with the absolute minimum temperature recorded over a thirty year period being -1.1°C (Figure 1). The soils in the area are typically granite-derived, deep, red ferrasols of the Hutton form (McVicar *et al.*, 1977).



**Figure 1.** The mean monthly rainfall (a) and maximum and minimum temperature (b) distribution recorded at Frankfort State Forest from 1959 to 1989. Graph (b) presents the absolute monthly maximum and minimum temperatures and also the mean monthly maximum and minimum temperatures.

### 2.2.2 HPV data analysis

In this thesis I have followed the method of Marshall (1958) and Swanson (1983), and applied the necessary correction for wounding (Swanson and Whitfield, 1981). This approach is process-based and does not require the calibration of the technique on the species on which it is to be used. Hence this chapter is aimed at verifying whether the application of theory developed by the above workers results in accurate sap flow estimates in *E. grandis*.

The verification experiments were carried out using a 'Custom Heat Pulse Velocity Recorder' (Soil Conservation Centre, P.O. Box 8041, Palmerston North, New Zealand). The system includes a data logger with four sets of probes, each of which give a point estimate of heat pulse velocity.

The four pairs of teflon probes with a single thermistor in each probe were connected in a Wheatstone bridge configuration and adjusted to zero output before generating the heat pulse. The heat pulse was generated by supplying a current to the four line heaters for 0.7 seconds, and the time taken for the bridge to return to the initial balance point after the heater was pulsed was then recorded.

The heat pulse velocity ( $u$ ) can then be calculated using the following formula (Swanson, 1983):

$$u = (X_u + X_d)/2t \quad (\text{m s}^{-1}) \dots\dots\dots [1]$$

where:  $X_u$  and  $X_d$  are distances (m) from the line heater to the upstream and downstream thermistors respectively. Both  $X_u$  and  $X_d$  are regarded as positive quantities.  $(X_u + X_d)/2$  is referred to as the probe separation in the remainder of this chapter. 't' is the time taken in seconds for the bridge to return to the initial balance point after the heater is pulsed.

Early HPV studies found that the technique consistently under-estimated actual sap flow by approximately half (Doley and Grieve, 1966; Cohen, Fuchs and Green, 1981). Swanson (1983) investigated the source of the under-estimation by simulated the effect of the non-conducting, wounded tissue, which develops as a result of probe implantation, on the transfer of heat to and from the sapwood. The result of the work was the publication of wound-correction co-efficients for a range of wound widths, and a selection of probe materials and espacements (Swanson and Whitfield, 1981). These were all found to influence the measurements of heat pulse velocity. The heat pulse velocity ( $u'$ ) was therefore corrected for the effect of wounding using Swanson and Whitfield's (1981) wound correction co-efficients for teflon probes.

The correction takes the form:

$$u' = p + qu + r(u)^2 \dots\dots\dots [2]$$

where: p,q and r are the correction coefficients derived for the measured wound size and teflon probes in the (-0.5,0,1.0) configuration.

The corrected heat pulse velocities are converted to sap flux using the formula of Marshall (1958):

$$v = \rho_b(m_c + 0.33)u' \quad (\text{kg m}^{-2} \text{ s}^{-1}) \dots\dots\dots [3]$$

where: v = sap flux

$\rho_b$  = wood density

$m_c$  = moisture fraction, dry weight basis

0.33 = specific heat of dry wood

The sap flow rate was calculated by multiplying the sap flux [3] by the sapwood area:

$$\text{Sap flow} = vA \quad (\text{kg s}^{-1}) \dots\dots\dots [4]$$

where: A = sapwood area

The moisture fraction in equation [3] was calculated using:

$$m_c = (\text{fresh weight} - \text{dry weight})/\text{dry weight} \dots\dots\dots [5]$$

In this study on *E. grandis*, the moisture content was measured in four sapwood samples from each tree which were first weighed fresh and subsequently dried in an oven at approximately 80°C until no further weight loss was recorded.

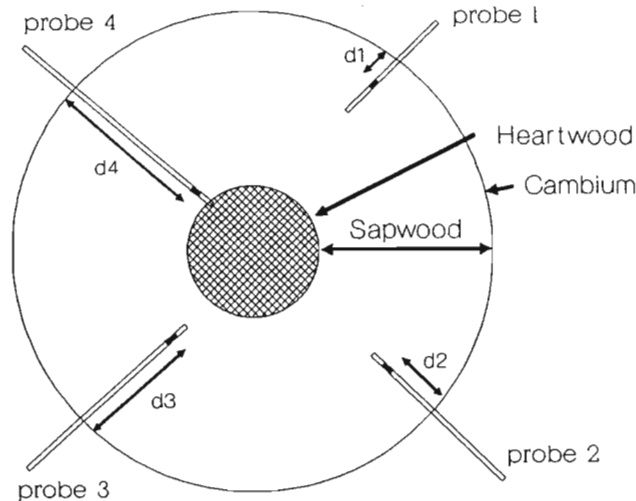
The wood density,  $\rho_b$ , in equation [3] was calculated using:

$$\rho_b = \text{dry weight/volume of fresh wood} \dots\dots\dots [6]$$

Archimedes' principle was used to determine the volume of the wood samples. This entailed recording the change in mass on the immersion of the wood sample in a beaker of water placed on a scale.

Because radial differences in sap flux do occur (Swanson, 1983), each of the four probes implanted to a different depth within the sapwood to ensure that the variation in the sapwood was covered (Figure 2). Each probe was therefore used to derive an estimate of sap flux considered to represent that position in the sapwood. The sapwood area associated with each probe was then calculated on the basis of four concentric rings with their limits mid-way between successive thermistors. Total sap flow was calculated as the sum of these partial areas multiplied by their associated sap fluxes. This method follows that of Swanson (1983) and Hatton *et al* (1990) is referred to as the 'step function' in this document.





**Figure 2.** A schematic diagram illustrating a cross-section through a stem and the probe implantation strategy used on *E. grandis*. d1 to d4 represent the increasing depths to which the single thermistor bead in each probe is implanted beneath the cambium.

### 2.2.3 Cut tree procedure

The HPV estimates of water use of one 16-year-old and four three-year-old trees were compared to a direct measure of water uptake recorded during a series of cut-tree experiments. This technique (Roberts, 1977) entails cutting a tree at its base and inserting it in a bucket of water. Verification involved measuring direct water uptake from the bucket and comparing this to uptake calculated from simultaneous HPV measurements.

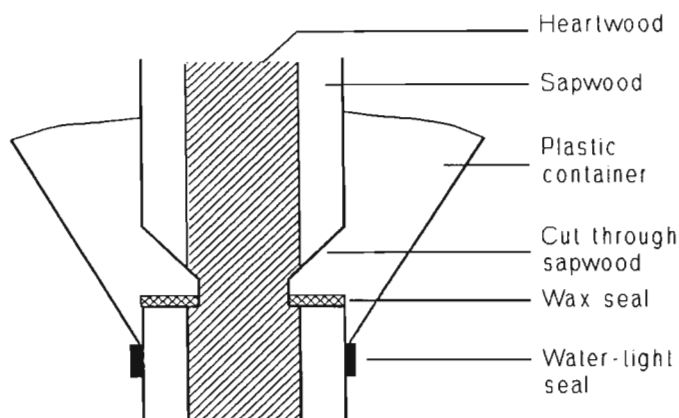
#### (i) *Three-year-old trees.*

The trees selected were typically 17 to 20 m tall, carried a leaf area of 50 to 80 m<sup>2</sup> and had a diameter at cambium of about 13 cm (at 1.3 m). Prior to cutting, the tree was tied to a supporting tripod and tethered to neighbouring trees for stability (Roberts 1977, Knight *et al.*, 1981). Immediately after cutting, the cut end was inserted in a bucket with water. The entire cutting procedure took approximately 30 seconds. Cutting was performed before dawn when water potential was highest, and the exposure of the cut end was kept to a minimum to prevent entry of air into the vessels.

An aqueous coloured dye was added to the water in the bucket to examine the pattern of water uptake in the sapwood. A trial run indicated the necessity of making a fresh cut under water with a sharp, broad-bladed chisel to prevent the occurrence of vessel blockages. This was shown to be successful since it resulted in an even distribution of dye throughout the sapwood. All bark was also removed from the section of the stem in the bucket to prevent the introduction of phloem exudates into the water (Green and Clothier, 1988).

*(ii) Sixteen-year-old tree.*

The tree selected was 56 m tall, had a diameter at cambium of 41.2 cm and 35.0 cm at the heartwood (at 1.3 m), and a leaf area of 219 m<sup>2</sup>. The cut-tree method was modified for the larger tree as a tripod support was not practical. Prior to cutting, the bark was removed from the basal section of the stem. A flexible, water-tight container was made by wrapping a plastic sheet around the stem and sealing it at the bottom and side where the sheet overlapped. The tree was left standing and a girdling cut (approximately 5 cm deep) made using a chainsaw through the sapwood to the heartwood around the entire circumference of the tree. The heartwood was thus left intact in order to support the tree. A second cut was made about 5 cm beneath the first and the section between the two cuts removed to facilitate access to the lower sapwood surface. This surface was then sealed with molten candle wax to prevent basipetal movement of water. The container was pulled downwards during cutting and raised and filled with water immediately the cutting had been completed. A metal strip was riveted to the upper rim of the container forming a rigid frame from which it was suspended in the raised position. This is illustrated below (Figure 3). The upper sapwood surface was then re-cut under water with a sharp chisel to remove any blockages. Water uptake from the container was measured by refilling it to a fixed mark every 10 or 15 minutes.



**Figure 3.** A diagram of the modified cut-tree method used on the sixteen-year-old tree.

#### **2.2.4 Wound size measurement**

On completion of each experiment the sections of the tree trunk containing the probe implantation holes were excised from the stem. Each sample was shaved with a microtome and the wound width measured with an ocular graticule on a dissecting microscope. The wound was identified as that section of the sapwood in which tyloses had been deposited in the vessels. Measurements were taken midway between the line heater element and both the upper and lower thermistors. Eight readings were taken along the length of each sample, making a total of 32 estimates for each set of four probes. A single average was then used as the final wound width estimate for each tree.

#### **2.2.5 Probe placement**

Preliminary investigation showed that the sapwood in both age classes of *E. grandis* is about 30 mm thick. The placement of probes was slightly off-set towards the outer part of the sapwood as this is where sap flux is highest (Chapter 5), and it accounts for a relatively greater sapwood area. The probe depths within the sapwood were thus set at 4, 10, 17, and 23 mm beneath the cambium for both size classes.

Each probe was implanted in a different quadrant around the circumference of the tree to account for variation around the stem. Deformities in the stem were avoided. In the larger tree, the outer surface was divided into eight sectors and a probe placed at a random position within each sector.

The probes were inserted through holes drilled radially in the stem at a height of approximately 1.3 m. The access holes were drilled with a battery operated hand drill fitted with a 1.85 mm drill bit. The probes were implanted in the (-0.5, 0, 1.0) configuration (Swanson, 1983). A 20 mm-thick drill jig was used to ensure that the holes were correctly spaced and parallel to each other. Following the experiments the precise probe separation was determined by inserting steel pins in each set of holes. The pins were then withdrawn from the holes with double-sided tape stuck to a card. This ensured that the original orientation of the pins was maintained. A line was then traced along either side of each pin on a second card. The distance between the heater and upper and lower thermistors (probe separation) was then measured at a point corresponding to the depth of the thermistors with a calibrated eyepiece.

#### **2.2.6 Measurement of the diameter at cambium and heartwood**

The salmon-pink heartwood of *E. grandis* is easily distinguished from the creamy-coloured sapwood. The distinction is enhanced by the application of methyl orange stain to a cross-section of the stem. This results in a crimson coloured heartwood through reaction with the acidic tyloses, and a neutral orange colour in the sapwood (Bamber and Fukazawa, 1985). The diameter of the outer limit of the sapwood, referred to as the diameter at cambium, and the diameter of the heartwood, was then taken as the average of four measurements on the section of the stem containing the HPV probes.

In order to check whether the area indicated as sapwood by the methyl orange stain was in fact indicative of the portion of the stem actually conducting water up the stem of the tree, a simple test was carried out during one of the cut tree studies. A dilute, coloured aqueous dye was added to the water in the bucket and the distribution of the

dye in a cross section of the sapwood compared to that produced when same sample was stained with methyl orange. The test revealed that the area delineated as sapwood by the methyl orange stain coincided precisely with that stained by the aqueous dye added to the water in the bucket. This indicated that the methyl orange stain could be used to accurately delineate the sapwood in *E. grandis*.

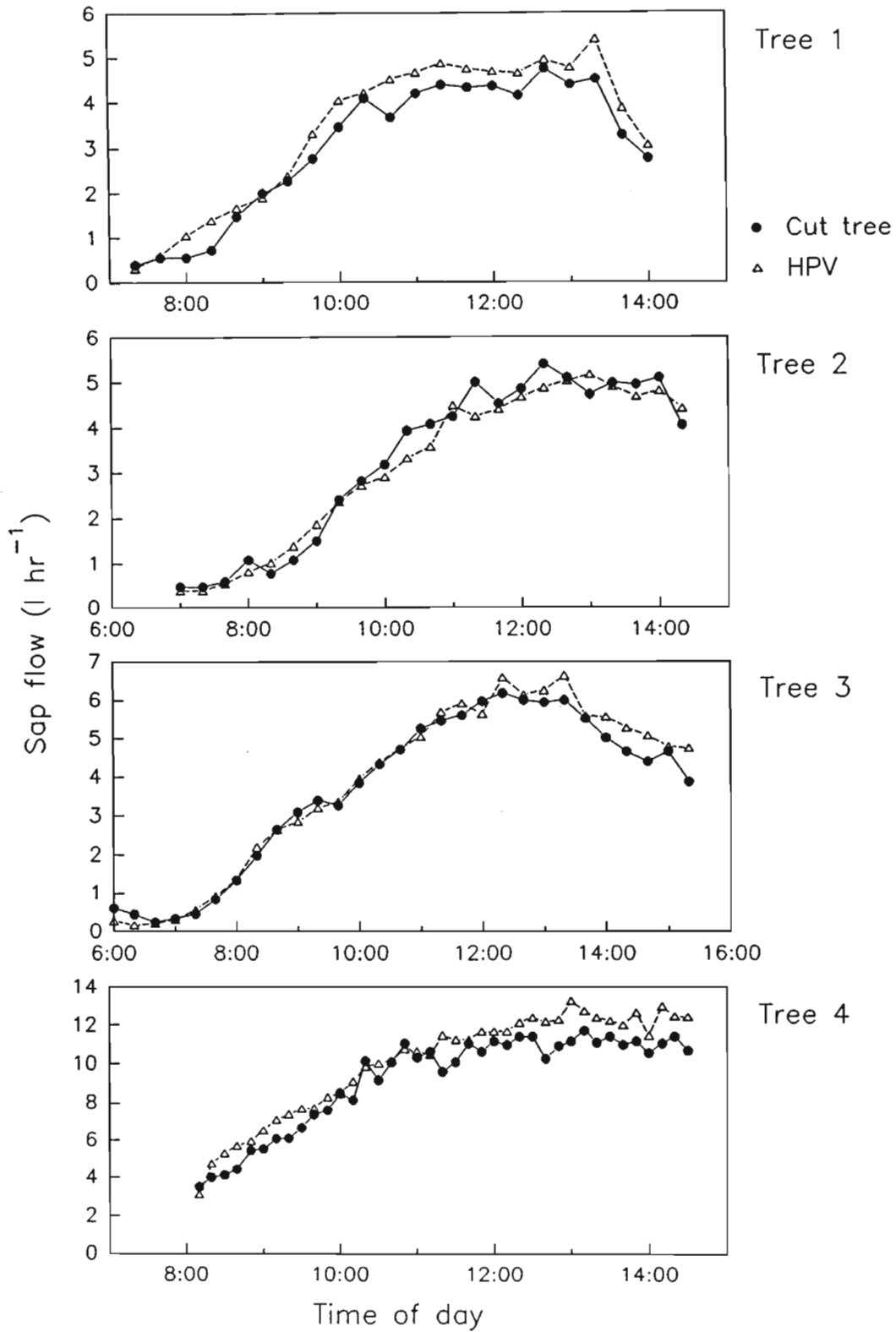
### **2.2.7 Sensitivity analyses**

These analyses were conducted through a mathematical simulation of the effect of errors in the measurement of the following parameters: probe separation, wound size, wood density and moisture content. These were selected as they were felt to be the most likely sources of error in the estimation of sap flow. The effect of increasingly serious errors in each of the above variables was calculated for a typical data set from one of the three-year-old trees.

## **2.3 RESULTS**

### **2.3.1 Verification on young *E. grandis* trees.**

Four cut tree experiments were conducted on trees between three and four years of age (Table 1). Sap flow estimated with the HPV technique corresponded well with the measured uptake of water from the bucket (Figure 4). This good correspondence was, however, very dependent on the accurate determination of wound size. At worst, the cumulative uptake recorded by the two methods differed by 12.7%, and at best 2.4%, with an average of 4.0% for the four trees.



**Figure 4.** A comparison of HPV and cut-tree estimates of sap flow through the course of a day conducted on four three-year-old *E. grandis* trees. The HPV values were corrected for the wound associated with the implantation of the thermistor and heater probes.

**Table 1.** The dimensions and leaf areas of the trees used in the verification of the HPV technique on *E. grandis*. The A-pan evaporation rates are given as indicators of the evaporative demand over the experimental period.

Tree no.	Age (years)	Height (m)	Diameter		Sapwood area (cm <sup>2</sup> )	Leaf area (m <sup>2</sup> )	A-pan (mm d <sup>-1</sup> )
			Cambium (cm)	Heartwood (cm)			
1	3	-	12.1	5.4	92.9	-	4.0
2	3	19.5	13.6	8.8	84.7	53.69	3.5
3	3	17.9	12.3	8.4	62.7	40.17	3.5
4	3	18.5	15.5	10.8	97.1	71.43	5.0
5	16	56.0	41.2	35.0	371.1	219.30	5.0

The moisture contents (Table 2) varied between sample trees, ranging from 1.26 to 1.96 with a mean of 1.64. The wood density was more constant ranging from 0.37 to 0.39 with a mean of 0.38. The measured wound widths varied considerably considering that both the drilling technique and the interval between probe implantation and measurement were relatively constant among the sample trees. This may have been due to some variation in the alignment of the probe holes in the vertical plane, rather than solely due to variations in the biological response to wounding.

**Table 2.** The moisture content, wood densities and wound widths of the five *E. grandis* trees used in the verification experiments.

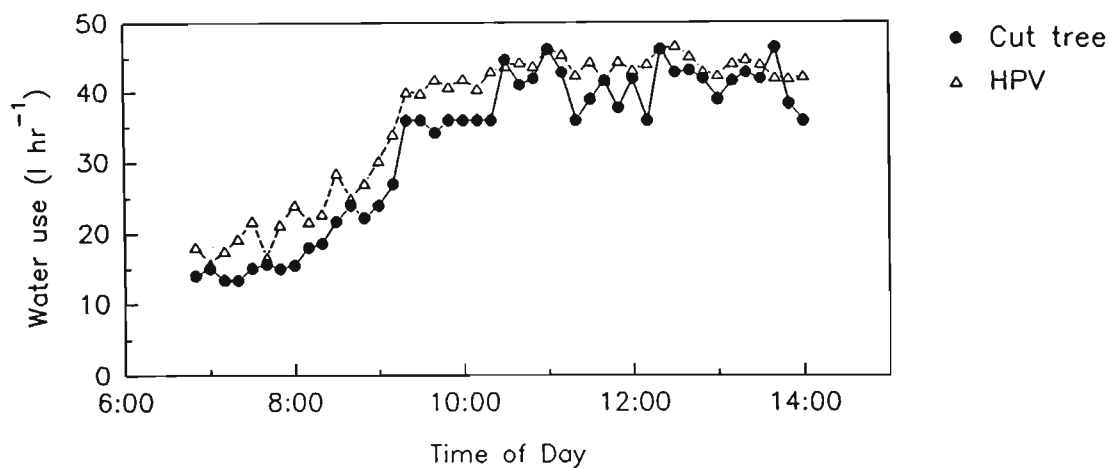
Tree	Age (years)	Moisture fraction $m_c$	Wood density $\rho_b$ (g cm <sup>-3</sup> )	Wound width (mm)
1	3	1.26 ± 0.083	0.38 ± 0.013	2.74 ± 0.388
2	3	1.96 ± 0.065	0.37 ± 0.006	2.33 ± 0.372
3	3	1.73 ± 0.089	0.38 ± 0.020	2.97 ± 0.318
4	3	1.73 ± 0.048	0.39 ± 0.006	2.81 ± 0.403
5	16	1.52 ± 0.091	0.42 ± 0.012	3.23 ± 0.237

It was of some concern that four probes may not adequately cover the variation in sap flux in the sapwood. However, the success of the four cut tree experiments conducted on young *E. grandis* trees indicates that four probes, each giving a single estimate of sap flux, were sufficient for the young trees. There are also physical limitations on how many probes one can insert in the stem of a small tree without either causing serious disturbance to sap flow, or, implanting the probes in undesirable positions.

### **2.3.2 Sixteen-year-old *E. grandis*.**

On completion of this study, a total of 238.8 l had been taken up from the bucket compared to a HPV estimate of 266.5 l - a difference of only 11.6%. The daily course of transpiration (Figure 5) shows that the HPV estimates were generally higher than the cut tree estimates, especially during the course of the morning, while the midday results compare more favourably. Difficulties were experienced in adding large quantities of water (40 l hr<sup>-1</sup>) to the flexible bucket in a short space of time. This resulted in the fluctuations in the cut-tree estimate of water use over midday. However, the correspondence between the two measures of water use was remarkably good considering that only eight probes were used on such a large tree. This corresponds to a sampling intensity of 1 probe per 46.5 cm<sup>2</sup> sapwood, while four probes in a small tree (13 cm in diameter with a 3.0 cm thick sapwood) corresponds to 1 probe per 23.6 cm<sup>2</sup>. The sampling intensity in the smaller trees was thus nearly twice that of the larger tree. This is a potential source of error which is best dealt with by sampling as intensively as practical.





**Figure 5.** A comparison of HPV and cut-tree estimates of sap flow through the course of a day conducted on a 60 m tall, sixteen-year-old *E. grandis* tree. The HPV values were corrected for the wound associated with the implantation of the thermistor and heater probes.

### 2.3.3 Sensitivity analyses.

The estimate of flux was particularly sensitive to errors in the measurement of wound size and probe separation, and relatively less sensitive to errors in the estimation of moisture content and wood density (Table 3). The response to errors in wound size and probe separation is non-linear, errors being relatively more serious when they are large than if they are small. For example, a 10% error in wound size results in an 18% error in flux, while a 50% error results in a 120% error in flux. Over-estimates also tend to result in more serious errors than underestimates of the same magnitude.

**Table 3.** The sensitivity of calculated flux to errors of varying magnitude in the measurement of selected variables.

Error in measured variable (%)	Resultant error in flux (%)			
	Wound size	Probe separation	Moisture content	Wood density
10	18.0	18.7	8.3	10.0
20	40.3	39.0	16.7	20.0
-20	-29.7	-32.6	-16.7	-20.0
50	120.3	109.6	41.9	50.0

## 2.4 DISCUSSION

Physically and anatomically, *E. grandis* is an ideal species for HPV work. The bark is generally thin (rarely thicker than 25 mm) and the thin sapwood (usually no thicker than 30 mm) means that long probes are not required. The absence of resin facilitates the insertion and removal of the delicate probes. Anatomically, *E. grandis* is a diffuse porous hardwood with approximately 8 vessels per mm<sup>2</sup> and vessel diameters ranging from 0.081 to 0.160 mm (Malan, 1988). Growth rings are either only vaguely evident or absent altogether (Malan, 1988). The estimation of wound size from excised sections of the sapwood is relatively simple, provided about five days have passed to allow adequate development of tyloses.

My initial attempts at verifying the accuracy of the HPV apparatus revealed very poor correspondence between the cut-tree and the HPV estimates of water use. A gradual decline in water-uptake was evident through the course of the morning despite increased levels of PAR and VPD. I therefore suspected that there was a problem with the cut tree procedure. Green and Clothier (1988) reported a similar decrease in the uptake of water due to the blockage of vessels which was remedied by re-cutting the

stem. Re-cutting *E. grandis* resulted in an immediate increase in water uptake which indicated that the vessels had been blocked and that it was necessary to take greater care to achieve a clean cut that would not affect the uptake of water from the bucket. Despite having solved this problem, and achieving a steady uptake of water from the bucket for the duration of the day, the HPV technique still continued to seriously under-estimate actual water uptake.

In searching for reasons for this lack of correspondence, it was found that the sapwood was evenly stained by the dye added to the water, except for the radial band where the flow of water was interrupted by the HPV probes. The width of this band ( $\pm 2.74$  mm) was invariably wider than the 1.85 mm drill bit used in the implantation of the probes. When the width of this band (rather than the drill size) was used as a measure of wound size, a good correspondence between the two estimates of water use resulted, implicating this as the major source of error. It was later found that the unstained band corresponded with tyloses deposited in the vessels, a process which takes about five days to occur. Hence wound size estimates in intact trees can be made from measurements of the band of tyloses on excised sections of the sapwood. The sensitivity analyses also indicate that it is important to measure this wound size accurately, particularly since the experimental data shows that wound size was on average 48% greater than the size of the drill used.

The response to wounding depends on season (Lopushinsky, 1986), phenological state (Shigo and Hillis, 1973), and increases with time after probe implantation (Swanson and Whitfield, 1981). These factors are probably largely responsible for the variation observed between the sample trees. This, together with the implications of a serious error in wound size, indicates that it is necessary to measure wound size with each implantation of probes. Unfortunately, the simpler system employed by Swanson and Whitfield (1981) in adding 0.4 mm to the drill size would not suffice in *E. grandis*.

The sensitivity to errors in the measurement of probe separation indicates that it is also necessary to take particular care over these measurements. Although the use of steel

pins was apparently adequate, direct measurements off excised sections of the stem containing the probe insertion holes would be more reliable (if destructive sampling is acceptable). This method would also be much easier to apply in trees where probes were deeply implanted, or in trees which exude resin.

A likely source of error is in the measurement of the moisture content, particularly if care is not taken to prevent the evaporation of water from the wood sample. Although this is a source of error, its potential magnitude is not as serious as that which can be made in the estimation of the wound size.

There are differences in the published literature regarding the sequence of calculations followed in converting heat pulse times to sap flow. This thesis follows the method and terminology of Marshall (1958) and Swanson (1983), where the correction for wounding (Swanson and Whitfield, 1981) is applied to the heat pulse velocities. However, Edwards and Warwick (1984) apply the wound correction to what they call sap velocity (equivalent to sap flux), while Green and Clothier (1988) apply it to sap flux directly. Since the correction is a function of velocity squared this can result in an under-estimation of sap flow by as much as 20%. Errors are particularly serious at high sap flow rates and large wound sizes. The approaches of Edwards and Warwick, and Green and Clothier, do, however give the same result as Swanson (1983), provided the wound corrections are applied to the heat pulse velocities rather than to sap flux.

Some HPV workers advocate fitting a quadratic function to the measurements made at four depths using a least squares statistical procedure (Edwards and Warwick, 1984; Green and Clothier, 1988). The measure of flux is then derived by integrating the area under the curve about the stem axis between the limits of the cambium and the sapwood/heartwood interface. Techniques generally in use assume the sap flux profile to be parabolic, but this is not always the case (Dye, *et al.*, 1991). Since the deepest and shallowest thermistors should not be placed at the inner and outer limits of the sapwood (Swanson, 1983), the calculation of total sap flow requires extrapolation beyond the limits of sampling. The use of a U-shaped best-fit function

therefore can result in a serious over-estimation of sap flow for the section of the sapwood beyond the limits of the deepest and shallowest thermistors. The most reliable method is thus to use a step function, each thermistor being representative of a certain section of the sapwood. Where the profile is approximately parabolic both the step function and the curve-fitting routine perform adequately. The advantage of using the simple step function, however, is that it performs well irrespective of the shape of the sap flux profile.

Sap flow has been measured in stems of trees using as few as one set of probes (Doley and Grieve, 1966), to as many as sixteen (Swanson, 1983). Most commonly either four (Edwards and Warwick, 1984; Green and Clothier, 1988) or six probes (Cohen *et al.*, 1981) are used per stem. Few have thoroughly examined how many are actually required, and where they should be placed in the sapwood for accurate estimates. Clearly, given enough insight into the variation in sap flux with depth in the sapwood along various radii, it is theoretically possible to derive accurate estimates of sap flow with a single strategically placed sensor. However, this information is generally not available beforehand and it is important to use enough probes to cover likely variation in the sapwood.

Swanson (1983) felt that 8 to 12 probes should be adequate in measuring sap flow in the stems of unstressed trees, but this should be increased to one per  $\text{cm}^2$  (sapwood) for stressed trees. The results presented for *E. grandis* suggest that four probes are probably adequate for young *E. grandis* trees (one probe for  $25 \text{ cm}^2$  sapwood). It is not possible to comment on the required sampling intensity on the older tree because only one tree was sampled. The sampling intensity used on the three-year-old trees coincides with that used by Swanson (1983). It would, however be advisable to determine the precise sampling requirements prior to fieldwork, using a technique such as outlined by Dye *et al.* (1991), since these may well differ between species and perhaps even growing conditions. This matter is discussed in more detail in section 5.3.3.

## 2.5 CONCLUSIONS

This study has shown that the HPV technique is particularly suitable to study water use by the diffuse porous hardwood, *E. grandis*. The technique accurately reflected water uptake in both age classes. The method does, however, demand particularly accurate wound size and probe separation estimates.

For the successful field application of the apparatus in *E. grandis* it is recommended that:

- (1) At least five days should pass between probe implantation and sampling to allow adequate time for tylosis development. This facilitates wound size measurement.
- (2) Particular care should be taken over the measurement of wound size and probe separation.
- (3) More than four probes be used on trees larger than about 20 cm diameter, whilst four probes are adequate for smaller trees, but this needs to be verified on the species to be studied.
- (4) A simple step function should be used to calculate total sap flow from each of the HPV point estimates.

## CHAPTER 3.

# TRANSPIRATION BY Eucalyptus grandis OF DIFFERENT AGES MEASURED USING THE HEAT PULSE VELOCITY TECHNIQUE

### 3.1 INTRODUCTION

Hydrological modelling in forest systems has been attempted by a variety of research institutes in a number of countries around the world. Foremost amongst these are the models developed in the USA by Running and co-workers (eg. Running and Coughlan, 1988), the Institute of Hydrology at Wallingford in Great Britain (Roberts, 1983a; Roberts, 1983b), and the CSIRO in Australia (Dawes and Hatton, 1993). None of these models include a component of tree age, although some models do allow one to modify the state variables with season or time (Running and Coughlan, 1988). The question of variation with tree age has not been addressed in current forest hydrological models. Two possible reasons for this are firstly, that until recently, instrumentation has not been available which allows transpiration to be measured from several trees during the course of a day. Secondly, much of the work done on catchment scale, either using gauged catchments or techniques such as eddy correlation, has been done in forest stands or catchments composed of a mixture of different tree age-classes with a complex of both understorey and overstorey trees contributing to evaporation.

In this section of the thesis I describe the application of the HPV apparatus in a series of field studies to address the following questions:

- i. Does transpiration from *E. grandis* vary with tree age?
- ii. Does a seasonal shift in the relationship between transpiration, and VPD and PAR (as found by Dye, 1987) also occur in a larger sample of trees?
- iii. What is the relative importance of climate, season and tree age in determining transpiration from *E. grandis* where soil water is not limiting?

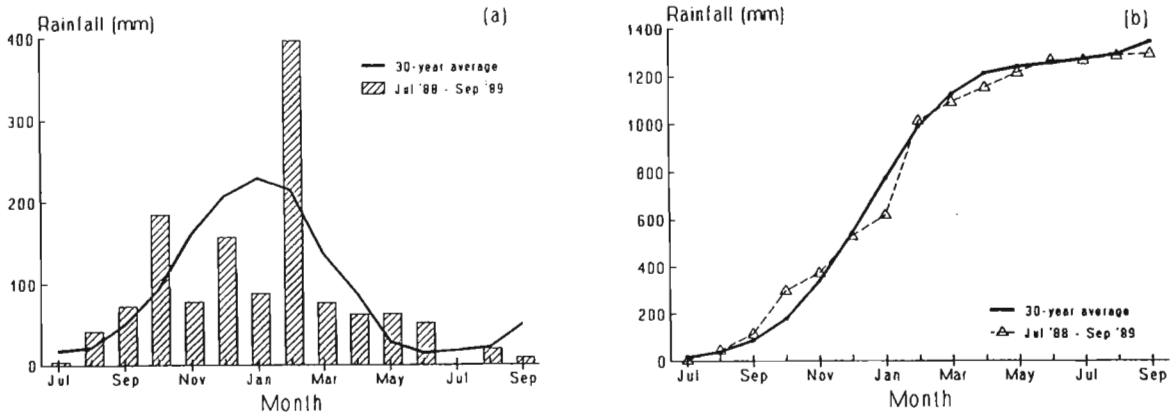
## 3.2 METHODS

### 3.2.1 Site description

This study was carried out on Frankfort State Forest (30°43' E, 24°49' S) in the Eastern Transvaal province of South Africa. All stands selected had a north-easterly aspect to minimise differences in the radiation load received by the stands. In order to minimise differences in the advected energy at each of the stands, no stands were selected immediately adjacent to unplanted areas. All the selected stands were a minimum of one hectare in size and consisted of an even-aged monoculture of *E. grandis* trees.

Frankfort State Forest is at an altitude of approximately 1100 m and receives a mean annual rainfall of 1230 mm per year, falling predominantly during the summer months from October to April (Dye *et al.*, 1990). Rainfall recorded during the experimental year is presented in Figure 6. The soils are granite-derived, deep, well drained and ferrallitic, and are classified under the Farninham series of Hutton Soils (McVicar *et al.*, 1977).





**Figure 6.** The monthly rainfall totals (a) and cumulative seasonal total (b) from July 1988 (the winter prior to the age sequence study) to September 1989 (when the age sequence study was concluded). For comparison, the thirty-year monthly average rainfall at Frankfort State Forest has also been presented.

### 3.2.2 Stand and tree selection

Five sample trees were selected from each of five stands of plantation-grown *E. grandis*, ranging from three to sixteen years of age. This covered the typical saw-log rotation in South Africa of about 20 years (Schönau and Stubbings, 1987). Stands were selected in the vicinity of the site studied by Dye (1987) for purposes of comparison. The silvicultural management of each of the stands is presented in Table 4. The trees in each stand were divided into five size classes on the basis of stem diameter, and one individual was selected at random within each size class.

**Table 4.** Compartments selected for the age-sequence study, their planting dates, densities, thinning regimes and mean annual stem volume increments.

Age (years)	Date of planting	Tree density at planting	Tree density after thinning (stems per hectare)						MAI $m^3ha^{-1}yr^{-1}$
			1st	Date	2nd	Date	3rd	Date	
3	Dec. 1985	800	-	-	-	-	-	-	-
6	Mar. 1983	1372	774	'85	517	'88	-	-	-
8	Jan. 1981	1372	721	'84	503	'86	-	-	52
13	Feb. 1976	1350	762	'79	487	'81	-	-	49
16	Jan. 1973	1350	742	'77	502	'80	301	'83	48

### 3.2.3 Heat pulse velocity sampling

The heat pulse velocity technique (HPV) was used to gain rapid whole tree estimates of transpiration. Probes were implanted in each of the selected sample trees at least five days prior to the field measurements. Four probes were used in each of the three-year-old trees and the smallest tree in each of the other age classes, while eight probes were used in all remaining older trees.

A preliminary survey showed that the bark thickness of the trees increases with tree age. The bark of the three-year-old trees was about 5 mm thick, while the bark of the sixteen-year-old trees was up to 25 mm thick. It was therefore necessary to tailor the probe implantation procedure to ensure that the probes sampled the entire sapwood.

The HPV logger was moved from tree to tree on a rotational basis during one day of intensive sampling in each stand during the wet summer season, and again towards the end of the dry winter. Sampling was done only on clear, sunny days, during which the canopy was dry. This was required to ensure that the measured transpiration rates were not confounded by evaporation from wet foliage and to avoid interruptions due to rain.

Simultaneously, PAR, relative humidity (RH) and temperature were recorded for modelling purposes. PAR was recorded in a clearing near each site, while the RH and temperature were recorded using a MCS<sup>1</sup> 174 sensor fitted with a Coreci capacitance sensor. Relative humidity and temperature were measured at approximately mid-canopy height in each stand. This was achieved by suspending the MCS 174 sensor beneath a tethered, helium-filled balloon, which was floated above the canopy. The RH and temperature sensors were connected to a MCS 120 data logger programmed to record instantaneous values synchronized with the HPV readings.

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<sup>1</sup> Mike Cotton Systems, P.O. Box 73, Steenberg, 7947, South Africa

The sample trees were felled within a week after sampling, and their leaves stripped and weighed fresh. Prior sampling had shown a good relationship between leaf area and fresh mass ( $1 \text{ kg} = 3.161 \text{ m}^2$ ) for a range of age classes (unpublished data). This relationship was used to determine the leaf area of the sample trees. At the same time, the moisture content of the sapwood, wood density, probe separation, wound sizes, and the diameter at cambium and heartwood were measured for the conversion of heat pulse velocities to sap flow.

#### *(i) HPV data collection and processing*

The same apparatus which was used in the verification of this technique on *E. grandis* was again used in this section of the fieldwork. The technique was applied and the data analyzed as outlined in section 2.2.2.

#### **3.2.4 Xylem pressure potentials**

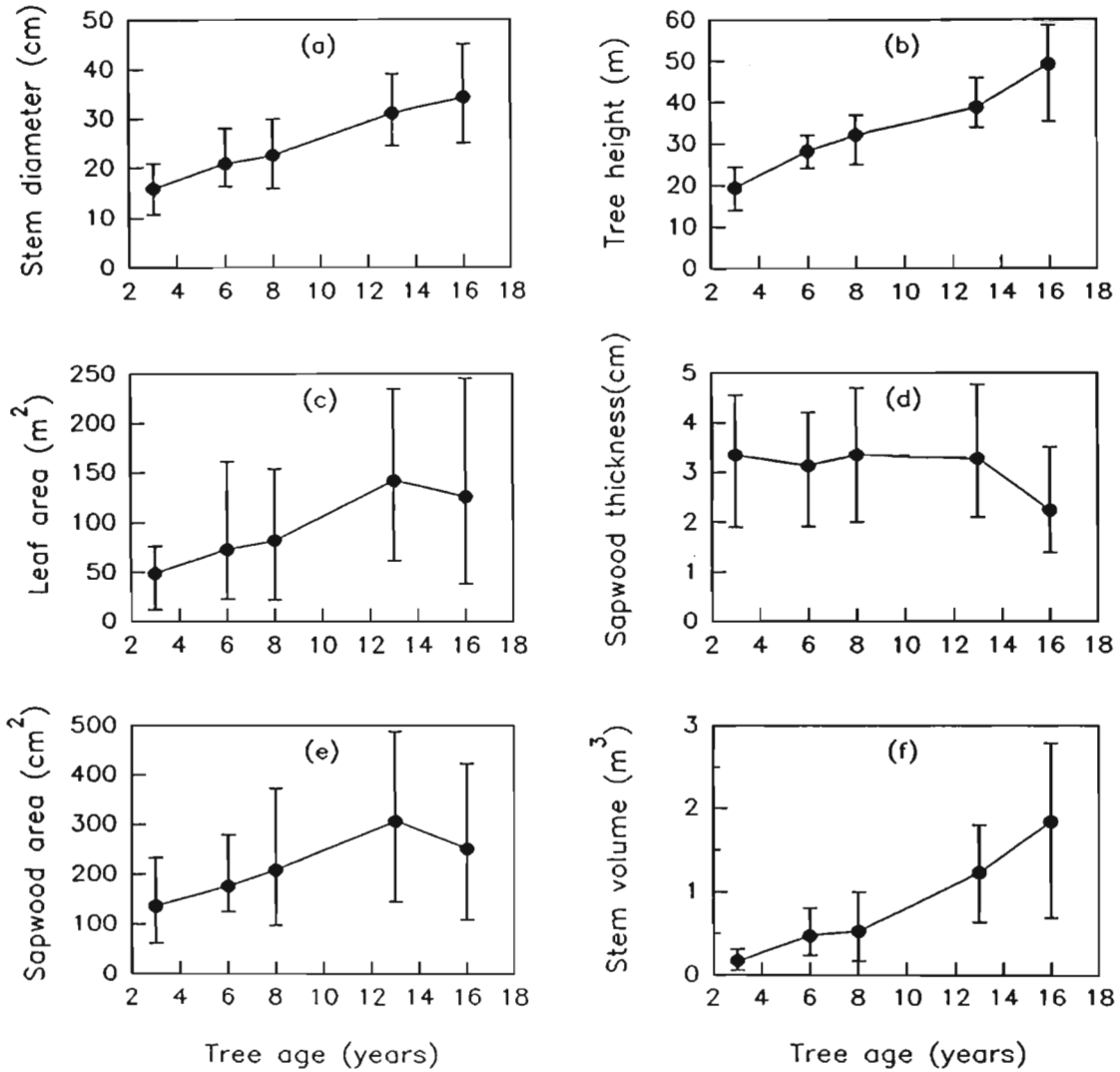
Pre-dawn and midday xylem pressure potentials were recorded for each of the sample trees on the day of intensive sampling. Leaves were removed with the aid of a cutting device raised to the canopy by a helium balloon (Poulter, Olbrich and Dye, 1991) and immediately inserted in a PMS pressure chamber.

### **3.3 RESULTS AND DISCUSSION**

#### **3.3.1 Morphological characteristics**

The mean tree heights, stem diameters, leaf areas and total sapwood areas (at breast height) of the sample trees exhibited a general increase with age (Figure 7). Although this is not surprising, it does indicate that the growing conditions on the selected sites were similar.

The mean height of the sixteen-year-old trees was almost 50 m, which emphasises the advantage of the heat-pulse-velocity instrument over techniques such as porometry, where the canopy must be reached for measurements to be made.



**Figure 7.** Changes in the mean and range of the diameters at breast height (a), heights (b), leaf areas (c), sapwood thicknesses (d), sapwood areas (e) and stem volume (f) with increasing tree age. The range, rather than the standard deviation, has been presented to give an impression of how much the parameters overlap between age classes.

The variation in the physical parameters between trees within a single age class was generally large. For example, the range of leaf areas (Figure 7c) and sapwood areas (Figure 7e) was large enough that there was considerable overlap even with the trees in the next older age class. This shows that, although the stem volume increased from year to year, leaf area and sapwood area were not as strongly governed by age, but probably reflect some other quality, such as water availability and light intensity on the site. Aspect could therefore be an important determinant of the site LAI.

Two points are worth emphasizing in Figure 7. Firstly, there appears to be a clear relationship between the way the leaf area and sapwood area change with age (Figure 7c and e). This will be explored in more detail in section 3.3.2. Secondly, the sapwood thickness of the sample trees (Figure 7d) was relatively constant in all age classes, with the exception of the 16-year-old trees. Also, the range of values encountered was consistent. This suggests that the rate of heartwood formation in these trees was fairly constant on the sites. Had one site been moisture stressed, one would expect heartwood formation to be accelerated. This is encouraging, as it indicates that any differences in the response of transpiration between the age-classes is not due to differences in site conditions.

The stem volumes in Figure 7f were calculated using the formula developed by Bredenkamp (1982) for *E. grandis*. Because stem volume is a function of both tree diameter and height, it is not surprising that the trend in height reflects that in diameter in Figure 7b.

### **3.3.2 Leaf area - sapwood area relationships**

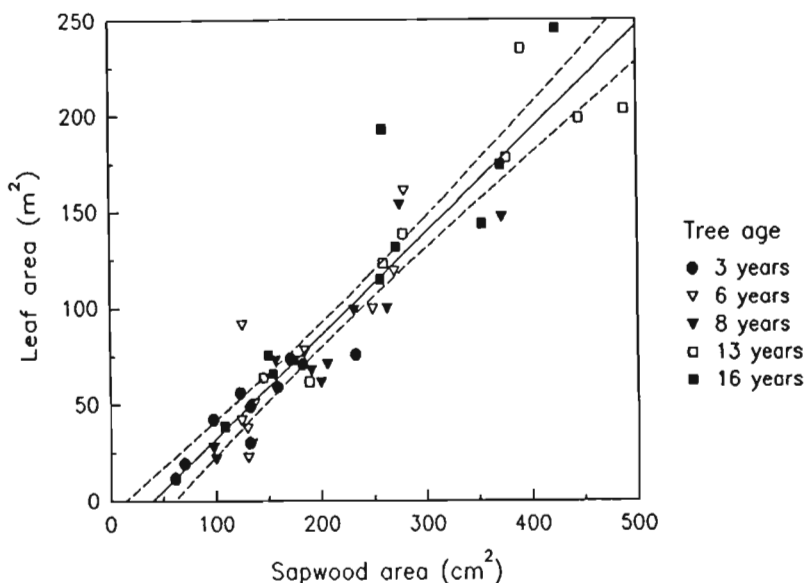
In this study it was possible to destructively sample each of the sample trees to determine their leaf areas. However, in future applications of the model it may not be possible to carry out such a sampling procedure, particularly if a large number of trees is involved. It would be valuable to know whether leaf area could be inferred from a more easily measured parameter, such as sapwood area.

The average leaf area per sample tree showed a general increase with age. In all cases the variation in leaf area within a single age class was large (Figure 7). Despite the large variation in leaf area, the leaf area and the sapwood area at breast height were found to be significantly correlated ( $R^2 = 0.87$ ;  $p=0.0001$ ). This relationship was consistent for all the trees sampled in a stand, whether dominant or suppressed, and for both the wet and the dry season. There was also no indication that the relationship between sapwood area and leaf area varied with age (Figure 8).

Although this general trend appears to be consistent, the range of leaf areas for any particular sapwood area is relatively large. This is best exemplified by the variation evident in the six-year-old trees (Figure 8).

There are two main sources of 'error' in these measurements. Firstly there is an experimental error in the sampling procedure due to the loss of leaves as the trees were felled. Although all efforts were made to minimise this error, it may still have occurred to some extent, particularly in the younger, denser stands. The second 'error' lies in the natural variation in the relationship between leaf area and sapwood area between individual trees in a stand. Recent work has shown that there is also considerable genetic variation in this relationship between clones of *E. grandis* (Olbrich and Poulter, 1992).

This implies that if leaf area were inferred from a measurement of sapwood area on an individual tree, the estimate of leaf area may be subject to a relatively large error. However, the relationship would be of value if one were to use it to estimate the total leaf area, or the leaf area index, of a group of trees or a stand. This could be achieved relatively easily with the aid of an increment corer and a suitable stain to define the limits of the sapwood.



**Figure 8.** The relationship between sapwood area at breast height and the leaf area of *E. grandis* trees three to sixteen-years-old. The best-fit regression has been plotted with its 95% confidence limits. The equation of the regression line is:  
 Leaf area (m<sup>2</sup>) = 0.5355\*Sapwood area (cm<sup>2</sup>) - 21.17 (r<sup>2</sup> = 0.87).

The relationship between leaf area and sapwood area for several conifers and hardwoods is summarised by Waring and Schlesinger (1985). A modified version of that table is presented in Table 5. The relationship varies both with species and environment. In general, trees with many large vessels (such as oak) support more foliage than trees with only small diameter vessels (Waring and Schlesinger, 1985). Also, more sapwood is required to support a given amount of leaf area in harsh environments.

The relationship for *E. grandis* was in the upper range of that recorded for hardwood trees, but not as high as that found for *Eucalyptus regnans* in Australia (Legge, 1985). However, *Eucalyptus regnans* is amphistomatous (Legge, 1985), which makes a direct comparison with *E. grandis*, a hypostomatous species, a little difficult. The comparatively high value for *E. grandis* probably reflects the combination of the benign Sabie climate (as indicated by the lack of water stress), and the presence of many relatively large vessels in the sapwood of this species (Malan, 1988). This implies that the sapwood of *E. grandis* is efficient at conducting water to the foliage. When *E.*

*grandis* trees are felled in the field, once the crown of the tree is removed water drains freely from the sapwood at the cut surface. This indicates that there is little resistance to the flow of water in the sapwood, supporting the above statement.

**Table 5.** A comparison of the relationship between sapwood area and leaf area for several hardwood trees.

Tree species	Leaf area/Sapwood area m <sup>2</sup> /cm <sup>2</sup>	Source
<i>Nothofagus solandri</i>	0.07	*
<i>Populus tremuloides</i>	0.10	*
<i>Nothofagus solandri</i>	0.12	*
<i>Acer macrophyllum</i>	0.21	*
<i>Quercus velutina</i>	0.25	Rogers and Hinckley (1979)
<i>Quercus alba</i>	0.40	Rogers and Hinckley (1979)
<i>Castanopsis chrysophylla</i>	0.46	*
<b><i>Eucalyptus grandis</i></b>	<b>0.54</b>	<b>This study</b>
<i>Tectona grandis</i>	0.65	*
<i>Eucalyptus regnans</i>	0.73	Legge (1985)

\* Source: Waring and Schlesinger (1985)

The utility of the relationship between leaf area and sapwood area lies most obviously in the relatively rapid determination of leaf area of either individual trees or even stands of trees. It is, however, important to realize that the relationship is fairly site specific (Waring and Schlesinger, 1985) and the relationship reported here should be used only on those sites with climatic and soil conditions similar to those in the Sabie area. A key factor here is the absence of water stress even during the dry winter months. Other relationships should be developed for sites which experience droughts. For example, Pook (1986) found that the leaf area index of Eucalypt forests alters dramatically in the event of severe droughts. It is also not clear to what extent the area of active sapwood changes under such conditions.



Further utility in the relationship between sapwood area and leaf area lies in its application in determining net primary productivity (Waring and Schlesinger, 1985). Growth rings are not generally visible in *E. grandis* in the Sabie area, but become more evident as a stronger seasonality is imposed through seasonal water stress, as occurs in some of the marginal forest sites.

### 3.3.3 Physiological characteristics

The most important factor limiting transpiration is the availability of water. One of the aims of this study was to study transpiration in the absence of water stress. For this reason pre-dawn water potentials were measured on the trees prior to the HPV sampling. These data are also relevant to the HPV sampling as increased water stress may lead to a more patchy transport of water in the sapwood (Swanson, 1983), which would introduce errors in sampling.

The HPV technique also requires that several measurements be made on sapwood samples from the sample trees. These include measurements of the moisture content, wood density and wound size. All these relate to the transfer of heat through the sapwood and are entered as constants in the equations outlined in Chapter 2. The moisture content and wood density can be determined either from increment cores, or larger sapwood samples taken from the tree. The determination of wound size, however, requires that a larger sample of wood be removed from the tree at the point where the probes were inserted. In most cases it is difficult to obtain sufficient material from the sample tree without sampling it destructively. In this section I will examine the variation in the above variables to establish whether they vary seasonally or with the age of tree.

#### (i) *Pre-dawn and mid-day xylem pressure potentials*

The pre-dawn pressure potentials (Table 6) indicate that water stress did not occur in any of the stands even towards the end of the dry winter season. There was some variation in the data, with the highest values being recorded for the sixteen-year-old

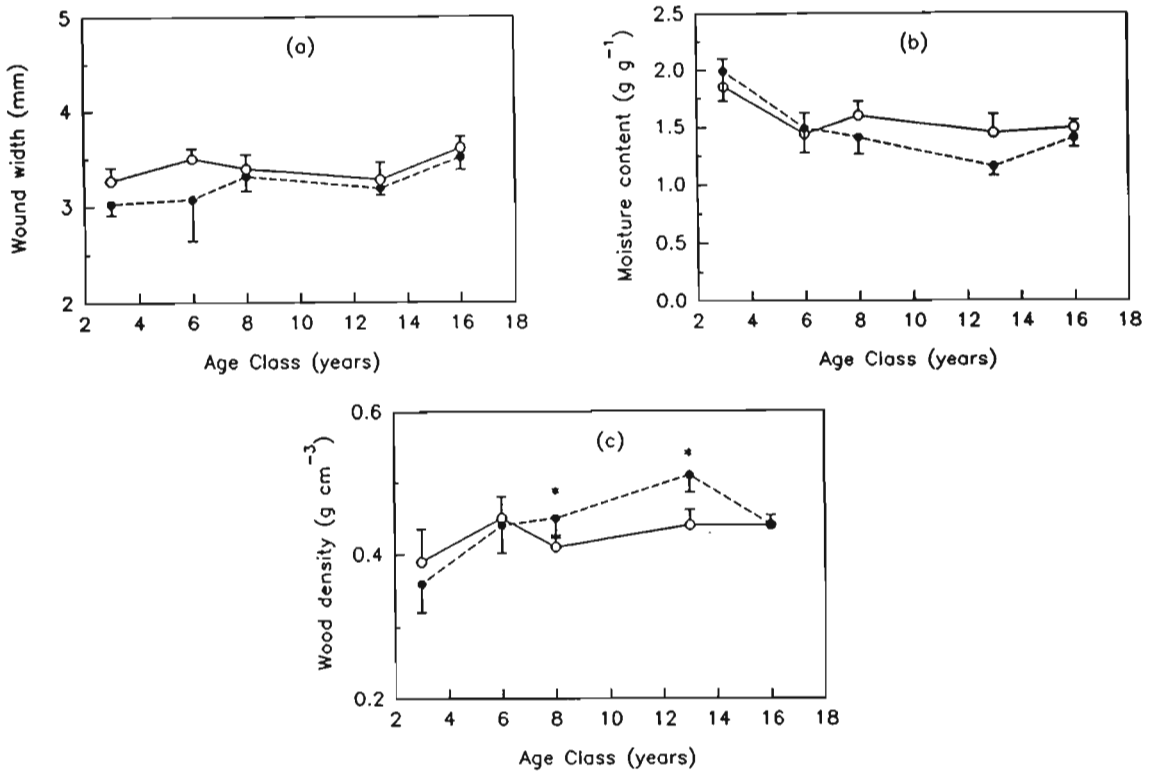
trees in winter. The midday values were generally lower than those recorded by Dye (1987). The indications are that the trees in the age-sequence study experienced a similar moisture regime to the individual studied by Dye (1987).

**Table 6.** Pre-dawn and mid-day xylem pressure potentials (MPa, mean  $\pm$  standard deviation) recorded for the sample trees in each age class during summer and winter.

Age class	Summer		Winter	
	Pre-dawn	Mid-day	Pre-dawn	Mid-day
3	-0.44 $\pm$ 0.11	-2.07 $\pm$ 0.20	-0.54 $\pm$ 0.16	-1.70 $\pm$ 0.19
6	-0.46 $\pm$ 0.13	-1.80 $\pm$ 0.28	-0.20 $\pm$ 0.03	-1.88 $\pm$ 0.12
8	-0.50 $\pm$ 0.14	-1.86 $\pm$ 0.24	-0.38 $\pm$ 0.11	-1.77 $\pm$ 0.17
13	-0.57 $\pm$ 0.11	-1.97 $\pm$ 0.36	-0.36 $\pm$ 0.06	-1.66 $\pm$ 0.25
16	-0.27 $\pm$ 0.09	-1.83 $\pm$ 0.27	-0.63 $\pm$ 0.08	-1.78 $\pm$ 0.21

**(ii) Trends in wound size**

In the verification section (2.3.3), I showed that the HPV technique was very sensitive to errors in the estimation of wound size. It would be convenient if it were possible to circumvent this measure, as the measurement of wound size is the most laborious of the follow-up data collecting procedures. Student's T-tests were conducted to compare the wound size measurements in summer to those in winter for each age class. The mean wound size was consistently larger in summer than in winter for all age classes (Figure 9), but the differences were not significant except for the three-year-old age class. If the data for all age classes are lumped together, the wound size in summer was significantly larger than winter (Table 7). This corresponds with the results of Shigo and Hillis (1973), who found that the wound response was suppressed during winter.



**Figure 9.** A comparison of the measured wound size (width in mm) (a), moisture content (b) and wood density (c) recorded in the sample trees in summer (○) and winter (●). The '\*' denotes statistically significant at the  $p=0.05$  level, while the error bars represent one standard deviation and are shown as one-sided for clarity.

### (iii) *Moisture content*

This variable quantifies the moisture content of the sapwood. Under drought conditions, the water content of the sapwood may decline as embolisms develop (Tyree and Sperry, 1991). Work done by Waring *et al.* (1979) has shown that certain Northern hemisphere trees, such as Scots pine, may draw on water stored in the sapwood to supply water for transpiration early in spring. This is reflected in changes in the sapwood water content. This may occur on a daily basis and, if rainfall is seasonal, also on a seasonal basis.

In this study, the highest moisture contents were recorded for the three-year-old trees, which also had the lowest wood densities (Figure 9). However, the moisture content remained fairly constant for the older trees, and was bound within a narrow range for

all ages (Figure 9). The seasonal difference was not significant (Table 7), nor were any of the differences within age classes significant. This corroborates the pre-dawn xylem pressure potential data (Table 6), which indicated that the trees experienced no water stress during either winter or summer.

**Table 7.** A comparison of the physiological characteristics of the sapwood measured in summer and winter on the trees sampled during the age-sequence study. Here the data within each age class have been lumped together to make the seasonal comparison with a Student's t-test. \*\* indicates significance at the  $p < 0.001$  level.

Variable	Season	Mean $\pm$ std	Significance
Wound size	Summer	3.41 $\pm$ 0.184	**
	Winter	3.22 $\pm$ 0.268	
Wood density	Summer	0.426 $\pm$ 0.025	N.S.
	Winter	0.440 $\pm$ 0.053	
Moisture content	Summer	1.57 $\pm$ 0.170	N.S.
	Winter	1.49 $\pm$ 0.305	

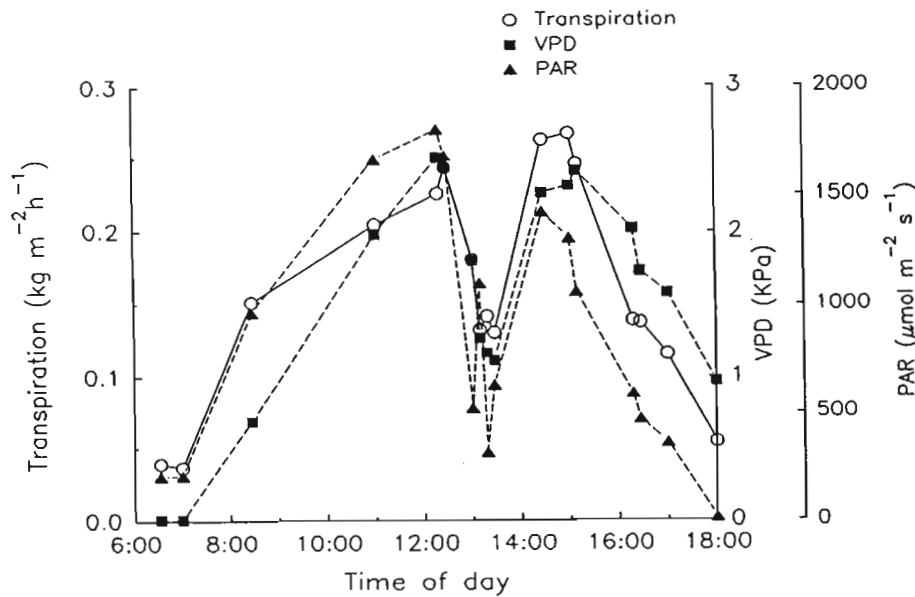
**(iv) Wood density**

The wood density should remain constant seasonally because *E. grandis* does not form growth rings (Malan, 1988). The apparent seasonal differences therefore describe the variation in wood density between trees within a stand. The sapwood densities were lowest in the three-year-old trees but were relatively constant for the remaining age classes sampled. The seasonal difference between the trees sampled from the eight and thirteen-year-old trees was found to be significant ( $p=0.05$ ). However, in general differences between summer and winter were not significant (Table 7).

### 3.3.4 The responsiveness of sap flow to changes in transpiration

The HPV technique was used to infer transpiration from measurements of sap flow rates at the base of the stem, which is remote from the actual site of transpiration, ie. the canopy. The canopies in some of the older *E. grandis* trees may be 50 m above the point where HPV readings are taken (Figure 7). This introduces the possibility of lags in the response of sap flow rates to changes in transpiration in the canopy. For example, Schulze *et. al.* (1985) recorded a 2-3 hour lag in *Picea* and *Larix* trees. Long lag times would make transpiration data collected with the HPV apparatus unsuitable for modelling instantaneous transpiration rates as I propose to do in this study.

The stomatal conductance, and hence transpiration from *E. grandis*, can be reliably predicted from PAR and VPD (Dye, 1987). During the course of the day on which the sixteen-year-old stand was sampled, a pocket of cloudy, moist air passed over the stand. This resulted in a rapid drop in VPD and PAR over the midday period (Figure 10). The findings of Dye (1987) predict that this should result in a concurrent decrease in the transpiration rate. Figure 10 shows that transpiration rate estimated using the HPV technique tracks the change in VPD and PAR through the midday period almost perfectly, with no apparent lag at the onset of the moister conditions. These data were collected from a sixteen-year-old *E. grandis* which was 58 m tall. Therefore, there appears to be no lag in the response to changes in sap flow resulting from changes in transpiration, despite the large separation between the site of HPV sampling and the canopy. In most cases the trees used in this study were smaller than the one used to illustrate this point, and therefore one can expect an even closer coupling between transpiration and sap flow. This indicates that HPV data collected from *E. grandis* is suitable for modelling instantaneous transpiration rates.



**Figure 10.** The response of transpiration (○) recorded with the heat pulse velocity apparatus to changes in the ambient vapour pressure deficit (■) and PAR (▲) in a 58 m tall, sixteen-year-old *E. grandis* tree.

### 3.3.5 Factors regulating transpiration

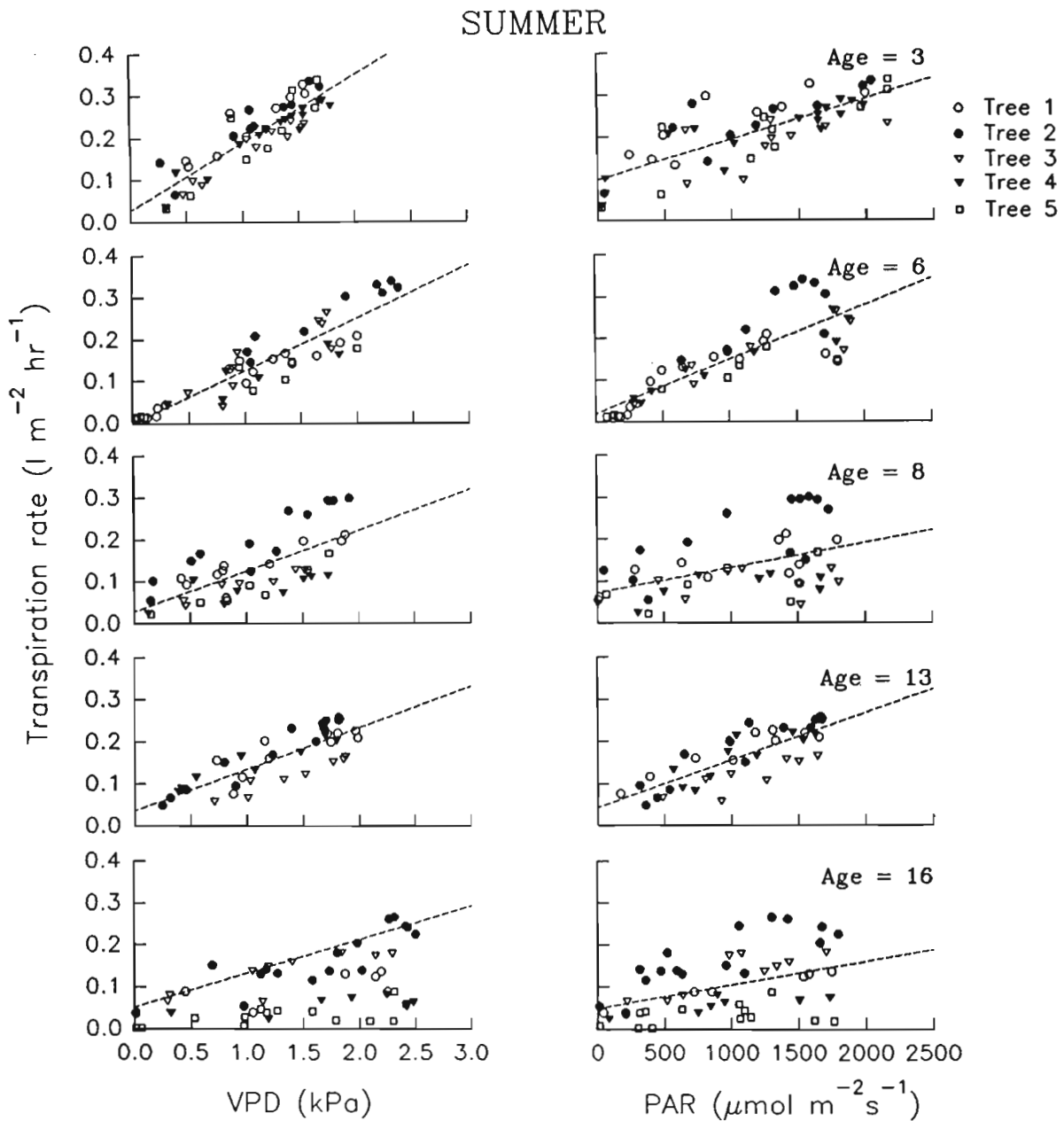
The collated data set contained a total of 556 estimates of instantaneous transpiration data with matching PAR and VPD data. This gives an average of 11.2 readings per tree, and 56 readings per age class per season. Given the analyses conducted in the sampling optimisation study, and the errors associated with the transpiration estimates for individual trees (5.3.3), only the mean response of the five trees sampled within each stand will be examined.

#### (i) Trends in the data

The two scatter plots of the data present a general overview of the relationships between transpiration and the two driving variables, PAR and VPD (Figure 11 and Figure 12). These graphs are intended to illustrate the general trends in the data and do not present the final relationships. To facilitate visual comparisons between age classes and seasons, the scale on the y-axis is the same in all the graphs presented.

The clearest trend is that the slope of the regression line decreases with age. This suggests that transpiration per unit leaf area declines with age, older trees transpiring less per unit leaf area than younger trees under equivalent climatic conditions. This trend was generally more consistent in summer than winter. Also, the slopes of the regression lines were generally steeper in summer than in winter (Figure 11 and Figure 12), which indicates that transpiration rates were lower in winter than under equivalent climatic conditions in summer. This finding is consistent with that of Dye (1987) who showed a seasonal change in the response of  $g_s$  to PAR and VPD.

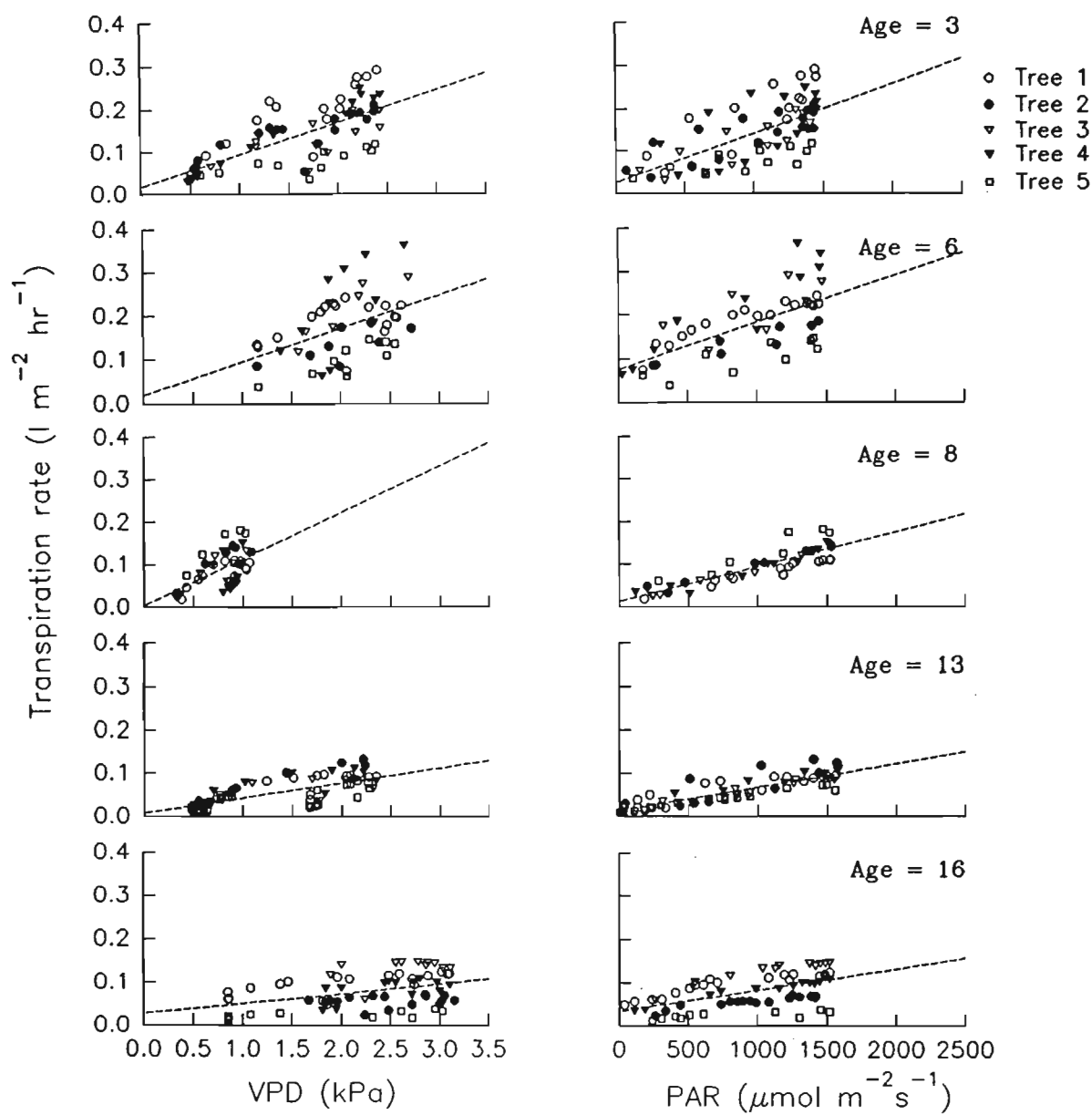
Although the scatter diagrams are useful in presenting the general trends, it is not possible to determine the response of transpiration to changes in either of the driving variables independent of the other. For example, the apparent response due to changes in VPD are confounded by simultaneous changes in the level of PAR. For this reason it was necessary to conduct further analyses, as reported in the following section.



**Figure 11.** Instantaneous transpiration rates measured using the HPV technique plotted against VPD (left column) and PAR (right column) recorded on the five sample trees in each age class. The figure reflects measurements taken during one day of sampling in each stand during **summer**. The dashed line represents the best-fit linear regression for the variables plotted.

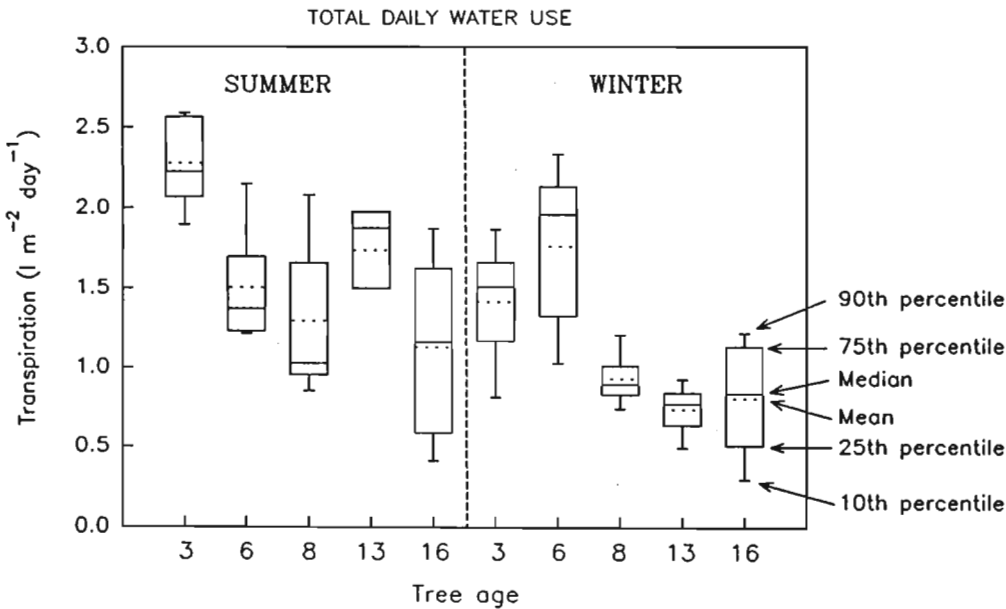


## WINTER



**Figure 12.** Instantaneous transpiration rates measured using the HPV technique plotted against VPD (left column) and PAR (right column) recorded on the five sample trees in each age class. The figure reflects measurements taken during one day of sampling in each stand during **winter**. The dashed line represents the best-fit linear regression for the variables plotted.

Daily total transpiration estimates were calculated for each of the sample trees (Figure 13). The data illustrate that the youngest trees generally transpired at faster rates than older trees, confirming the trend evident in the regression lines in Figure 11 and Figure 12. There are, however, two apparent anomalies: the thirteen-year-old trees in the summer data set, and the six-year-old trees in the winter data set (Figure 13). Despite these anomalies, there is a fairly clear trend towards a decline in transpiration with increasing tree age in both summer and winter. The figures portrayed in this section do not take account of climatic variation between the days on which the respective stands were sampled. This is examined in the following section.



**Figure 13.** A box and whisker plot showing the mean (---), median, 10th, 25th, 75th and 90th percentiles of the total daily transpiration recorded for each age class during summer and winter.

**(ii) Modelling the relationship between transpiration and the climatic variables**  
 Relatively few studies have modelled the relationship between climatic variables and transpiration. Most of the work done to date has centred on modelling stomatal conductance or canopy conductance. Transpiration rates are then calculated from the modelled canopy conductances using the Penman-Monteith equation (Dye, 1987;

Running and Coughlan, 1988; Whitehead and Kelliher, 1991). This approach has developed primarily from restrictions placed on the experimental approach by equipment available, most of the work cited above having been done with the use of porometers. This study is relatively unique because transpiration was measured directly. This removes the necessity of modelling stomatal conductance at several levels in the canopy, scaling up to the whole canopy, and then calculating transpiration. However, as stomatal functioning is at the source of the process, the relationship between stomatal conductance and the climatic variables should give an indication of how transpiration and the environmental variables are related. This can then be used to choose an appropriate function to model the latter relationship.

Dye (1987) showed that the two most important climatic factors regulating stomatal conductance were PAR and VPD. The relationship between stomatal conductance and PAR was shown to be hyperbolic, the stomata reaching light saturation at a level of about  $300 \mu\text{mol m}^{-2} \text{sec}^{-1}$ . VPD and stomatal conductance were inversely related, stomatal conductance decreasing with increasing VPD. The Penman-Monteith equation calculates transpiration as a linear function of stomatal conductance (Jarvis, 1986), and one would therefore expect the above relationships to hold for the dependency of transpiration on these environmental variables.

The approach followed to determine the best model reflects that suggested by Weisberg (1985). A subset of the data was abstracted to determine the nature of the function relating VPD and PAR to transpiration. The aim was to select trees from the extremes of the age-sequence to establish whether the same function could be used throughout, and whether the nature of the function changed from summer to winter. The subset was selected to contain data from one tree in each of two age classes in summer and winter. The criteria for selection were that the individual trees should have many data points, and as large a range of VPD and PAR through the course of the day as possible. The subset selected for summer comprised the data collected on tree 4 in the three-year-old age class, and tree 2 in the thirteen-year-old age class. The winter data were represented by trees 3 and 1 from the three and thirteen-year-old age classes respectively.

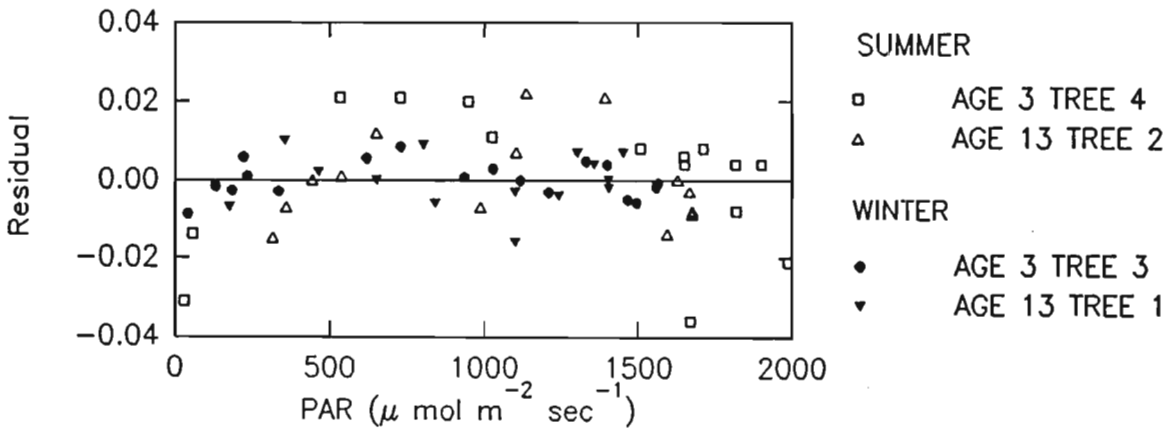
VPD and PAR separately accounted for almost 80% of the observed variation in transpiration in the four sample trees (Table 8). The best two-factor models accounted for approximately 97%, or a further 18% of the observed variation in transpiration. Adding a third factor in the model accounted for only just over 1.3% of the remaining variation. The best two factor model was one relating transpiration to a function of VPD and log(PAR). However, this model accounted for very little more variation than a straightforward multiple linear function of VPD and PAR. Taking the logarithm of PAR has the effect of linearising the response of transpiration to increasing PAR. This indicates that some non-linearity was evident in the relationship between transpiration and PAR, but that the curvilinearity was not pronounced.

**Table 8.** Correlation coefficients recorded for the best one, two and three-factor models tested to describe the relationship between transpiration and VPD and PAR.

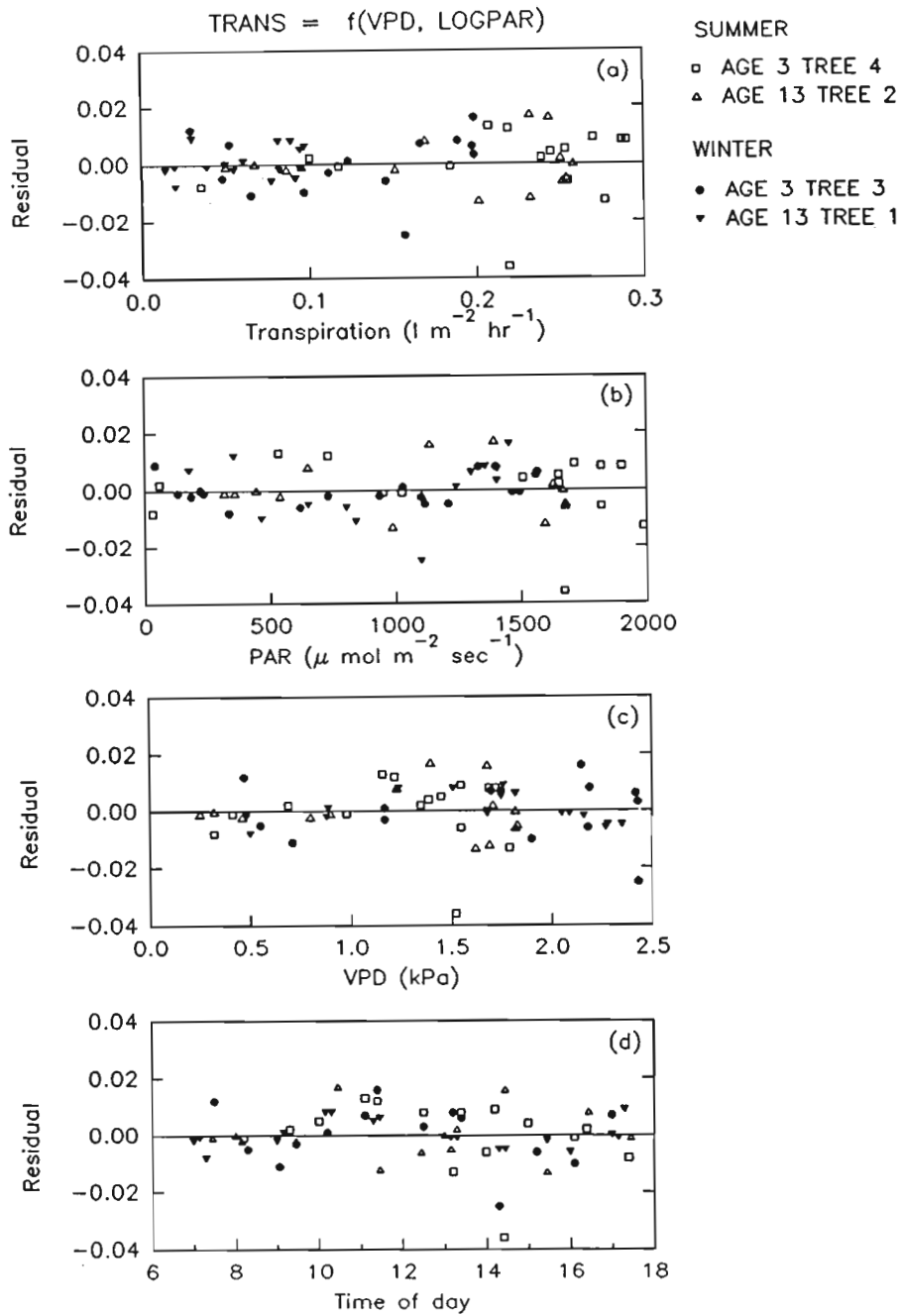
REGRESSORS	SUMMER		WINTER		AVERAGE R <sup>2</sup>
	Age 3, tree4	Age 13, tree 2	Age 3, tree3	Age 13, tree 1	
PAR	0.77	0.86	0.76	0.80	0.791 ± 0.048
VPD	0.93	0.94	0.70	0.55	0.782 ± 0.188
VPD, Log(PAR)	0.97	0.99	0.97	0.97	0.972 ± 0.009
VPD, PAR	0.94	0.98	0.98	0.98	0.968 ± 0.021
VPD, Log(PAR), PAR <sup>2</sup>	0.98	0.99	0.99	0.99	0.983 ± 0.006
VPD, PAR, Log(PAR)	0.98	0.99	0.98	0.99	0.983 ± 0.005

The model developed by Dye (1987) showed a non-linear relationship between changes in VPD and PAR and stomatal conductance. It was therefore important to check that the residuals of the best models cited in Table 8 did not show any curvilinear tendencies when plotted against predicted transpiration, VPD and PAR. The residuals of the regression of transpiration on PAR and VPD did show a moderate curvilinear tendency when plotted against PAR (Figure 14). This resulted in a systematic under-estimation at low levels of PAR and an overestimation of transpiration at mid-range PAR levels. The log transformation of the PAR term corrected for this tendency, resulting in an unbiased distribution and constant variance of residuals over

the range of recorded data (Figure 15). The model,  $Transpiration = f(VPD, \log(PAR))$ , adequately accounts for the non-linearity in the response of transpiration to PAR for individual trees. In contrast, the response to VPD was linear over the range of recorded data (Figure 15). There was no difference in the residual distribution between seasons, indicating that the nature of the response did not change seasonally. There was also no pattern in the residual distribution plotted against time of day (Figure 15), further substantiating that the response of sap flow to changes in evaporative demand is very quick, with no apparent lags due to capacitance.



**Figure 14.** The distribution of residuals plotted against PAR from the regression of transpiration on VPD and PAR.



**Figure 15.** The distribution of residuals plotted against predicted transpiration (a), VPD (b), PAR (c), and time of day (d) for the best two factor model of the form:  $Transpiration = f(VPD, \text{Log}(PAR))$ .

The evidence presented above confirms that VPD and PAR are the two most important driving variables regulating transpiration in *E. grandis* growing under conditions of non-limiting soil water. The simplest, most appropriate function describing the relationship

between instantaneous transpiration and the two driving variables was found to be of the form:  $Transpiration = f(VPD, \log(PAR))$ . This function was found to account for an average of 97% of the observed variation in transpiration in the four selected sample trees (Table 8). In addition, it was evident that the nature of the function was similar in both seasons. The influence of season is examined in detail in the following section.

**(iii) Variation in transpiration with season.**

A repeated measures analysis of covariance with random effects was conducted to determine the relative importance of the various factors in determining transpiration from the age sequence of trees. The analysis revealed that the four factors mainly responsible for controlling transpiration from *E. grandis* were: season ( $F_{1,555} = 216.1$ ;  $p=0.001$ ), VPD ( $F_{1,555} = 203.9$ ;  $p=0.001$ ), Log(PAR) ( $F_{1,555} = 130.5$ ;  $p=0.001$ ) and tree age ( $F_{4,555} = 77.3$ ;  $p=0.001$ ) in order of significance.

A least squares means analysis was used to compare transpiration rates within each age class and between seasons to determine whether there was a significant seasonal difference in transpiration. The analysis revealed that transpiration rates were significantly higher in summer than in winter in all age classes bar the six-year-old age class (Table 9). This implies that the rates of transpiration were generally higher in summer than under equivalent conditions of evaporative demand in winter. Overall, the summer transpiration rates were greater than the winter transpiration rates irrespective of age-class, with the exception of the sixteen-year-old trees (Table 9).

The anomalous nature of the six-year-old age class is also apparent in the box-and-whisker plot (Figure 13). The transpiration rates were the highest recorded in the winter months, and its ranking relative to the other age classes changed from summer to winter. The observed difference was possibly due to two factors: firstly the stand was thinned in the period between the summer and winter readings. This would have the effect of increasing light penetration to the lower branches of the sample trees. This could be expected to raise the transpiration rates from those branches (Waring and Schlesinger, 1981). Secondly, the pre-dawn xylem pressure potentials recorded

on the sample trees were the lowest of those recorded in winter (Table 6). The weather that morning was misty with slight drizzle. This has subsequently been shown to affect the pre-dawn xylem pressure potentials (XPP), resulting in lower values than one might expect (unpublished data). The lower XPP may then result in a feed-forward response, causing elevated transpiration rates and overriding the apparent downward seasonal trend.

**Table 9.** A least squares means analysis to determine whether seasonal differences in transpiration ( $l\ m^{-2}\ hr^{-1}$ ) within age-classes are significant. Means with the same letter are not significantly different at  $\alpha=0.05$ .

Age	Least squares means $\pm$ se	
	Summer	Winter
3	0.204 $\pm$ 0.006 a	0.133 $\pm$ 0.005 de
6	0.154 $\pm$ 0.006 bc	0.160 $\pm$ 0.006 cd
8	0.132 $\pm$ 0.006 bc	0.103 $\pm$ 0.006 ef
13	0.153 $\pm$ 0.006 b	0.072 $\pm$ 0.005 g
16	0.118 $\pm$ 0.006 f	0.063 $\pm$ 0.005 h

**(iv) Variation with age**

A repeated measures analysis with covariates was then run to test for the significance of age within each season. This revealed that tree age was significant in both summer ( $P < 0.0005$ ) and winter ( $P < 0.0004$ ), indicating the necessity of including a parameter in the transpiration model reflecting tree age.

The summer data show that the three-year-old trees transpired significantly faster than the six, eight and thirteen-year old trees (Table 9). The differences between the six, eight and thirteen-year old trees were not significant. This group, however, transpired significantly faster than the sixteen-year-old trees ( $p=0.001$ ). It should be emphasised that the transpiration rates have been normalised for leaf area and have units of  $l\ m^{-2}_{leaf\ area}\ hr^{-1}$ .



The data collected towards the end of winter shows a similar trend with a slight change in the order of the ages. The six-year-old trees transpired significantly faster than the three-year-old trees ( $F_{4, 555}$ ;  $p=0.001$ ), which in turn, were significantly faster than the eight-year-old stand. The slowest transpiration rates were again recorded on the oldest trees, the thirteen- and sixteen-year-olds.

The general trend in transpiration therefore indicates that the highest transpiration rates were recorded by the younger trees (three- and six-year-olds), the lowest transpiration rates were recorded by the sixteen-year-olds, while the eight- and thirteen-year-old trees were intermediate. There appears to be either a physical change, such as a decrease in stomatal density or aperture, or some physiological change which results in a decrease in transpiration potential with age.

### **3.3.6 The model relating transpiration to the two main driving environmental variables, PAR and VPD**

The above analyses have elucidated the nature of the function relating transpiration to the climatic variables PAR and VPD. They have also demonstrated the importance of including an additional variable, AGE, into the analysis when predicting transpiration from *E. grandis* in the Sabie area. It is important to emphasise that this situation is perhaps a little unique in that the trees were not found to be moisture limited, so the model presented effectively predicts potential transpiration from *E. grandis*.

The variety of multiple regression models tested (Table 10) can be placed in two groups. The first treats tree age as a continuous variable, the resultant models being of the form *Transpiration* =  $f(\text{Age}, \text{VPD and PAR})$ . In the second group, the models were developed for each of the age classes separately. In both cases, separate models were developed for the summer and winter data.

**Table 10.**

Multiple regression models relating transpiration from *E. grandis* under conditions of non-limiting soil water to the two driving climatic variables. The table presents the significance of the parameters tested, the significance of the models, and the adjusted R<sup>2</sup> of the various models tested. The number of \*'s are used to denote the level of significance: \*\*\* = 1%, \*\* = 5%, and \* = 10%.

Number of variables in the model	Season	Significance of parameters					Adj R <sup>2</sup>	Mean Adj R <sup>2</sup>	Prob > F
		Age	VPD	Log(PAR)	PAR	Intercept			
3	Summer	***	***	***	-	ns	0.69	0.71	***
	Summer	***	***	-	***	***	0.73		***
	Winter	***	***	***	-	ns	0.64	0.68	***
	Winter	***	***	-	***	***	0.72		***
4	Summer	***	***	*	***	***	0.73	0.73	***
	Winter	***	***	***	***	ns	0.72		***
2	Summer	3	***	***	-	*	0.88	0.73	**
	Summer	6	***	ns	-	ns	0.86		***
	Summer	8	***	**	-	ns	0.62		***
	Summer	13	***	**	-	ns	0.81		***
	Summer	16	***	**	-	ns	0.48		***
	Winter	3	***	***	-	**	0.66	0.60	***
	Winter	6	ns	***	-	**	0.55		***
	Winter	8	**	***	-	***	0.61		***
	Winter	13	***	***	-	***	0.76		***
	Winter	16	ns	***	-	*	0.41		***
	Summer	3	***	-	ns	ns	0.87	0.75	***
	Summer	6	***	-	***	ns	0.88		***
	Summer	8	***	-	***	ns	0.63		***
	Summer	13	***	-	***	ns	0.83		***
	Summer	16	***	-	***	ns	0.53		***
	Winter	3	***	-	***	ns	0.76	0.72	***
	Winter	6	***	-	***	ns	0.62		***
	Winter	8	**	-	***	ns	0.86		***
	Winter	13	***	-	***	ns	0.89		***
	Winter	16	ns	-	***	*	0.47		***
3	Summer	3	***	ns	**	ns	0.88	0.75	***
	Summer	6	***	***	ns	ns	0.88		***
	Summer	8	***	ns	ns	ns	0.62		***
	Summer	13	***	**	ns	ns	0.83		***
	Summer	16	***	**	ns	ns	0.52		***
	Winter	3	***	***	ns	ns	0.76	0.72	***
	Winter	6	ns	***	ns	ns	0.62		***
	Winter	8	**	***	ns	ns	0.86		***
	Winter	13	***	***	ns	ns	0.88		***
	Winter	16	ns	***	ns	ns	0.47		***

All the models presented in Table 10 were highly significant. The inclusion of both a PAR and a Log(PAR) term in the three-term polynomial models tested resulted in no significant increase in the amount of variation explained. Analyses presented earlier in this chapter on individual trees suggested that the best model would be of the form  $Transpiration = f(VPD, Log(PAR))$ . However, where the mean response of the five trees in the stand were examined together, this is no longer as clear. For example, the regression models in which the logarithm of PAR was used on the winter data, consistently returned a poorer  $R^2$  than those where the logarithm was not used (Table 10). In fact the models of the general form  $Transpiration = f(PAR, VPD)$  consistently accounted for more of the observed variation than those where log(PAR) was introduced as one of the terms. Models with PAR in its untransformed state generally returned a higher level of significance on the PAR term than those with Log(PAR). Also, where PAR, rather than log(PAR), was used in the model, the multiple regressions tended to have intercepts that were not significantly different from zero, a desirable feature because one would expect transpiration to be very low under conditions with low light and evaporative demand.

The best models therefore appear to be of the following forms:

1. Where age is treated as a continuous variable, an average of 72.5% of the observed variation was accounted for by a model of the form:  
 $Transpiration = f(AGE, VPD, PAR)$ .
2. Where the age-classes and seasons are treated individually, an average of 73.5% of the variation was accounted for by a model of the form :  
 $Transpiration = f(VPD, PAR)$ .

It should be noted, however, that this model results in more than 80% of the variation being accounted for in five of the ten cases tested (Figure 15).

Which of the two models is used depends on the purpose of the application. Obviously, questions of a specific nature such as "what is the transpirational loss from a three year-old eucalypt stand?" should be addressed using the model which treats the age-classes individually. Were the question more general, such as "What is the expected hydrological effect of shortening rotation times in *E. grandis* stands?" the utility of having a simple model statement including a function of tree age is of obvious benefit.

The best-fit multiple regression models are presented in (Table 11). The upper two rows of the table present the models where age was treated as a continuous variable, while the lower rows present the regressions where each age class was modelled separately for each season. The regression equations suggest that the response to VPD may be the mechanism responsible for the change in transpiration with age. This is illustrated by a systematic decrease in the coefficient of the VPD term in the regression equations (Table 11) from the young to the old trees. The coefficient of the VPD term was also generally greater in summer than in winter. By contrast, the coefficient of the PAR term does not show any systematic variation through the age sequence or by season.

One possible explanation for the age effect being related to the VPD response is that as the trees grow older there may be an enhanced feed-forward response to VPD. In other words the slope and/or the constant of the curve describing the relationship between canopy conductance and VPD, declines with age. This change may be mediated through a change through changes in the osmotic potential of the leaves, and may be similar to the mechanism responsible for the observed seasonal effect.

**Table 11.** The coefficients of the best models representing the relationship between transpiration ( $\text{l m}^{-2} \text{ hr}^{-1}$ ) and age (years), VPD (KPa) and PAR ( $\mu \text{ mol m}^{-2} \text{ sec}^{-2}$ ). Because of its anomalous nature, the data for the six-year-old age class in winter has been excluded from the analysis where age was treated as a continuous variable.

Season	Coefficients $\pm$ SE				R <sup>2</sup>	Prob > F
	Age	VPD	PAR	Intercept		
Summer	$-5.81 \times 10^{-3} \pm 6.52 \times 10^{-4}$	$6.62 \times 10^{-2} \pm 6.35 \times 10^{-3}$	$5.82 \times 10^{-5} \pm 6.79 \times 10^{-6}$	$0.0626 \pm 0.00813$	0.73	0.0001
Winter	$-5.54 \times 10^{-3} \pm 3.99 \times 10^{-4}$	$2.46 \times 10^{-2} \pm 2.70 \times 10^{-3}$	$5.72 \times 10^{-5} \pm 4.32 \times 10^{-6}$	$0.0573 \pm 0.00539$	0.72	0.0001
Summer	3	$15.1 \times 10^{-2} \pm 1.72 \times 10^{-2}$	$1.86 \times 10^{-5} \pm 1.29 \times 10^{-5}$	$0.0146 \pm 0.0105$	0.87	0.0001
	6	$9.24 \times 10^{-2} \pm 1.25 \times 10^{-2}$	$4.45 \times 10^{-5} \pm 1.39 \times 10^{-5}$	$-0.00447 \pm 0.00846$	0.88	0.0001
	8	$8.60 \times 10^{-2} \pm 1.38 \times 10^{-2}$	$3.40 \times 10^{-5} \pm 1.24 \times 10^{-5}$	$0.00271 \pm 0.0144$	0.63	0.0001
	13	$6.40 \times 10^{-2} \pm 1.41 \times 10^{-2}$	$6.09 \times 10^{-5} \pm 1.65 \times 10^{-5}$	$0.0141 \pm 0.0103$	0.83	0.0001
	16	$3.35 \times 10^{-2} \pm 1.11 \times 10^{-2}$	$5.68 \times 10^{-5} \pm 1.65 \times 10^{-5}$	$0.00596 \pm 0.0134$	0.53	0.0001
Winter	3	$4.97 \times 10^{-2} \pm 7.12 \times 10^{-3}$	$7.83 \times 10^{-5} \pm 1.13 \times 10^{-5}$	$-0.0151 \pm 0.0105$	0.76	0.0001
	6	$3.68 \times 10^{-2} \pm 1.33 \times 10^{-2}$	$9.50 \times 10^{-5} \pm 1.82 \times 10^{-5}$	$0.0102 \pm 0.0201$	0.62	0.0001
	8	$2.62 \times 10^{-2} \pm 1.10 \times 10^{-2}$	$7.31 \times 10^{-5} \pm 6.45 \times 10^{-6}$	$-0.000226 \pm 0.00623$	0.86	0.0001
	13	$1.61 \times 10^{-2} \pm 2.13 \times 10^{-3}$	$4.32 \times 10^{-5} \pm 2.74 \times 10^{-6}$	$0.000642 \pm 0.00253$	0.89	0.0001
	16	$0.782 \times 10^{-2} \pm 5.77 \times 10^{-3}$	$4.75 \times 10^{-5} \pm 9.90 \times 10^{-6}$	$0.0179 \pm 0.00975$	0.47	0.0001

### 3.3.7 Discussion

The analyses presented in this chapter confirm the findings of Dye (1987) that VPD and PAR accounted for a large proportion of the observed variation in transpiration. This is also in agreement with the predictions of Jarvis and Stewart (1979) that VPD and PAR would be the most important meteorological variables driving transpiration in tall, well ventilated trees.

It could be argued that it would have been more correct to model the transpiration process as a function of net radiation. However, the net radiation data would have been very difficult to obtain for the stands of *E. grandis* because it would have been necessary to place a net radiometer above the canopy of the trees, requiring a tall tower in each stand. Also, net radiation data are not readily available, therefore models developed with net radiation as an input variable are less useful in the applied world. By contrast, PAR data are easily attained and available from a network of automatic weather stations. Net radiation may have been theoretically more appropriate,

however, the purpose of the development was to address an applied problem in which data availability was one of the considerations that was addressed.

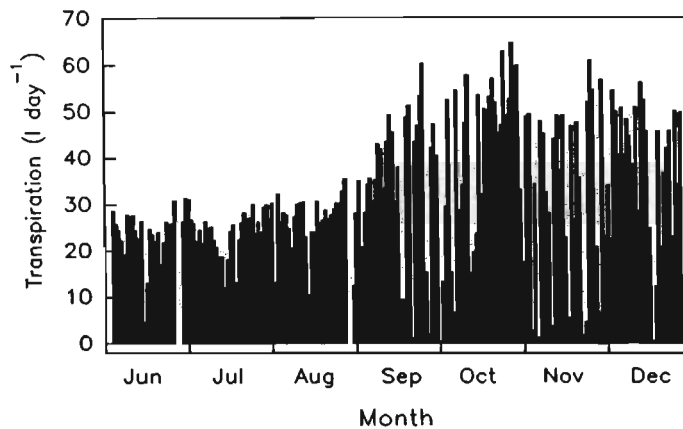
There was also a seasonal shift in the relationship of transpiration to the driving variables. One possibility is that this reflected a change in water availability, because the seasonal shift corresponded to the change from the wet summer months to the dry winter months. However, the pre-dawn and midday xylem pressure potentials indicated that the trees did not experience moisture stress, even towards the end of the dry winter season (Table 6). It would therefore appear that transpiration from *E. grandis* in this area is not limited by water availability, at least for the duration of this study. It is believed that this is due to the large reservoir of soil water available in the deep soils on the site. This has been corroborated by two studies (Dye, 1987; Dye, 1993a) which have found soils to be particularly deep. Dye and co-workers, using seismic techniques, measured soil depths of approximately 40 m, and found live root fragments at a depth of 20 m.

Further corroborative evidence has come from a study in which it was attempted to induce water stress in *E. grandis* trees by preventing the infiltration of rainwater into the soil (Dye, 1993a). Even after a full year of treatment the sample trees showed only very mild symptoms of water stress, pre-dawn xylem pressure potentials being elevated by only 0.3 MPa. It was also interesting to observe that the bulk of the water requirements of eight-year-old trees was met by uptake from depths in excess of 9 m in the soil.

This rooting pattern indicates that the trees are able to avoid moisture stress during the dry winter months by the extraction of water from deep sub-soil layers. Since the seasonal shift cannot be accounted for by a change in water availability, it implies that some underlying physiological mechanism is responsible for the shift. A change in osmotic potential from summer to winter may occur, resulting in lower transpiration rates during winter than under equivalent conditions during summer. Such seasonal osmoregulation was recorded for *E. regnans* (Connor *et al.*, 1977). However, a study to examine the cause of this shift has yet to be carried out.

Year-long sap flow data on a single three-year-old *E. grandis* tree illustrated a sharp rise in transpiration rates at the end of September (Figure 16). These results were recorded by P.J. Dye and are unpublished. This change occurred over a period of days, suggesting a physiological change triggered the response. The LAI and pre-dawn xylem pressure potentials, were stable over the period during which the change was recorded, ruling these factors out as causes of the change. The dramatic rise in transpiration rate from mid- to end-September (Figure 16) could also not be accounted for by increases in evaporative demand alone, further indicating the involvement of a physiological trigger in the seasonal change.

Soil temperatures are likely to undergo a seasonal change as a result of the natural seasonal change in ambient temperatures (Figure 1b). This may hold the key to the seasonal response as Waring and Schelessinger (1985) report that the root membrane resistance to water uptake may change with changes in soil temperature. This results in the inhibition of water uptake at low soil temperatures. In northern temperate climates species also do not bud break until soil temperatures rise above a threshold that does not inhibit water uptake (Waring and Schlessinger, 1985). A similar mechanism may operate in *E. grandis* regulating the seasonal uptake of water, giving rise to the observed seasonal change in transpiration rate.



**Figure 16.** Total daily sap flow recorded on a three-year-old *E. grandis* tree at Frankfort State Forest from June to December 1992 (this figure was reproduced with permission of P.J. Dye - unpublished data).

A change in transpiration with age in *E. regnans* was also recorded by Jayasuriya *et al.* (1993) in a catchment near Melbourne in Australia, although their time-frame was somewhat extended by comparison to this study. They demonstrated a decrease in transpiration ( $\text{mm ha}^{-1}$ ) from stands of trees from 50 to 230 years of age. Although they also demonstrated a decrease in sapwood area ( $\text{m}^2 \text{ha}^{-1}$ ) with stand age, which presumably translates into a change in leaf area with age, it is unfortunately not possible to interpret their results with respect to changes in transpiration per unit leaf area with age. They also do not comment on what may be causing the observed change with age.

The observed differences with age and season appear to have a shared origin. In both cases, the trend was most clearly evident as a change in the slope of the response function to VPD. The seasonal response could be due to a change in the osmotic potential of the leaf mesophyll cells, as was found in *Eucalyptus regnans* by Connor, *et al.* (1977). Alternatively, it could also be due to a change in the resistance of the flow path with low resistances in summer in the newly formed xylem and higher resistances in winter due to the cumulative effect of embolisms that develop during summer. It could be hypothesised that this seasonal change in resistance causes



signals to be sent to or accumulate in the leaves, that change the response of stomata to VPD .

A similar mechanism may be responsible for the observed change in transpiration with age, whereby the resistance in the flow path is increased due to the ever increasing contribution of catastrophic embolisms in the older branches. Young trees exhibit a self-pruning habit because their height growth is rather dramatic. However, as the trees grow older, their branches tend to become longer-lived as a result of the stands being progressively thinned as part of a standard management practice. The net result is that the older trees tend to have older branches with more tortuous flow paths, and therefore perhaps enhanced feed-forward responses to high VPD.

Cole *et al.* (1990) postulate that the resistance to water flow through the plant determines the conditions under which moisture stresses may develop. As such, tree size is almost certainly an important factor in determining the level of resistance experienced. They hypothesise that as trees grow taller the resistances to upward flow increase. The result is that the foliage of large trees will experience a degree of moisture stress, and consequently "the transpiration flux will decrease as the tree ages". However, they do not substantiate their argument with data or further references.

The data expressed so far have been on the basis of transpiration per unit leaf area per unit time. To extend these results to the level of the stand, one must incorporate an understanding of the dynamics of the leaf area index in *E. grandis* stands over time. A survey of *E. grandis* leaf area indices (LAI) in this area revealed that the LAI attains a maximum of approximately 4 by the age of three, which is maintained at this level until about age ten, after which it steadily declines to about 2 at the age of 25.

Considering both the changes in LAI and the expected changes in transpiration with tree age, we would expect the transpiration rate of a stand of *E. grandis* trees to peak early in the rotation, and remain high until approximately the age of ten, after which it will decline steadily as the trees grow older. Short rotations of about eight years are

commonly used for the production of pulp and mining timber. These will therefore have a greater hydrological impact than the longer saw log rotations (typically 20 - 25 years). A more detailed simulation is presented in the next chapter.

The above paragraph is potentially flawed in the respect that although the LAI of *E. grandis* trees may decline with age, it is not clear how the LAI of the stand changes in time. Field observations suggest that understorey development in *E. grandis* stands is fairly insignificant while the stands are young (less than 10 years old), but that understorey with a LAI at least in excess of one may develop as the stand is thinned and gaps open in the canopy. In such situations, the understorey vegetation would consist of a mix of self-seeded *E. grandis* saplings and a diversity of weed plants including *Solanum mauritianum* and *Setaria megaphylla*. Increased transpiration from the understorey vegetation would therefore offset decreases in transpiration from the *E. grandis* trees as the stands grew older.

A further point that should be raised is that the models developed in this chapter have used daytime meteorological data only. The nature of the models developed from these data have been of the form  $Transpiration = f(VPD + PAR)$ , hence the application of these models to night-time data would predict transpiration as a function of VPD. This may be erroneous, and further work need be done to determine to what extent night-time transpiration occurs in *E. grandis*.

In conclusion, the heat pulse velocity apparatus has been shown to be a valuable technique for water use studies on tall, fast-growing trees such as *E. grandis*. Transpiration may be modelled, without the necessity of accessing the canopy, as sapwood area provides a reliable estimate of canopy leaf area. This work has indicated that *E. grandis* appears to undergo a physiological change as it ages, such that its water usage is greatest in the early stages of the growth cycle. Also, transpiration rates were found to be higher in summer than under equivalent conditions of evaporative demand in winter.

## CHAPTER 4.

# VERIFICATION AND APPLICATION OF THE DERIVED TRANSPIRATION MODELS

### 4.1 INTRODUCTION

It is of some concern that the results achieved in the age sequence study are based on a single day's measurement on each stand in both summer and winter. The possibility exists that the nature of the function relating transpiration to the driving environmental variables may not be generally applicable in longer term predictions. Also, the seasonal shift may be a result of the fact that the data were collected from a single day in each stand. The question therefore arises as to whether these observations are also applicable to longer term data sets.

In order to ensure that the relationship between transpiration and the two key climatic variables, VPD and PAR, was represented accurately, the nature of the response function was determined from a much larger data set drawn from a water use efficiency study on four-year-old *E. grandis* trees growing on the same site (Olbrich *et al.*, 1993). The study necessitated continuous water use and growth measurements from selected trees over a six month period from December (early summer) 1990 to the end of May (late autumn) 1991. This provided a data set which could be used to test whether the seasonal effects and the nature of the function relating transpiration to the driving environmental variables was consistent with that observed in the age sequence study.

Also, it was of interest to establish how realistic the transpiration estimates from the best models developed in the age sequence chapter were. To this end, the best

models were applied to a sample meteorological data set collected at the Sabie Forestry Research Centre.

## 4.2 METHODS

The strategy used in the water use efficiency study to derive a continuous record of the transpiration rates from twelve selected four-year-old *E. grandis* trees was to measure the water use from one tree, the reference tree, continuously, and measure the other trees intermittently. The data for the one continuously measured *E. grandis* tree was used in this examination.

### 4.2.1 Sap flow measurement

Sap flow measurements were made at half-hourly intervals using the 'Custom' HPV apparatus for the duration of the study. Four HPV probes were implanted in the stem of the selected individual, which was 12.3 cm in diameter. Prior to probe implantation, a survey was conducted to determine the approximate sapwood thickness of the selected clone. 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 probes were implanted to the desired depth in each of the twelve trees and remained implanted over the period of the study.

The HPV data were processed in the conventional manner described in the methods section in the Chapter 2. This provided a continuous record of the sap flow rates from the selected tree over a six month period. The mean hourly transpiration was calculated from successive half-hourly measurements to synchronise the transpiration data with the hourly average meteorological data. Only daytime data were used because the resolution of the HPV technique is inadequate to measure sap flow rates at the very low rates commonly encountered at night time. For this reason, those data

where the hourly average PAR was less than  $50 \mu \text{ mol m}^{-2} \text{ sec}^{-1}$  were eliminated from the analysis. Days with rainfall were not excluded from the data set.

#### **4.2.2 Meteorological conditions**

The prevailing weather conditions were recorded at an automatic weather station 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<sup>2</sup> 120 data logger programmed to calculate hourly average PAR, RH and temperature. The vapour pressure deficit (VPD) was calculated from the humidity and temperature data.

#### **4.2.3 Statistical analyses**

Two blocks of data representing summer and autumn were selected for further analysis. A 29-day period was selected to represent 'summer' conditions from the 24th of December 1990 to the 21st January 1991. This period was selected because it represented the range of conditions normally experienced during summer, ie. a blend of cloudy days interspersed with the occasional hot, clear or partly cloudy day. The autumn data selected was a period 20 days long from the 3rd of May to the 26th of May 1991. The resultant models were tested on two independent data sets. These data were collected from the 22nd of January to the 18th of February 1991, and from the 15th of April to the 2nd of May 1991.

Regression analyses were conducted on these data to determine the most appropriate model relating mean hourly transpiration to the driving climatic variables. These were

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<sup>2</sup> Mike Cotton Systems, P.O. Box 73, Steenberg, 7947 South Africa

subjected to the screening tests suggested in Weisberg (1985), and again simple linear models were tested first.

## **4.3 RESULTS**

### **4.3.1 Meteorological conditions and transpiration rates**

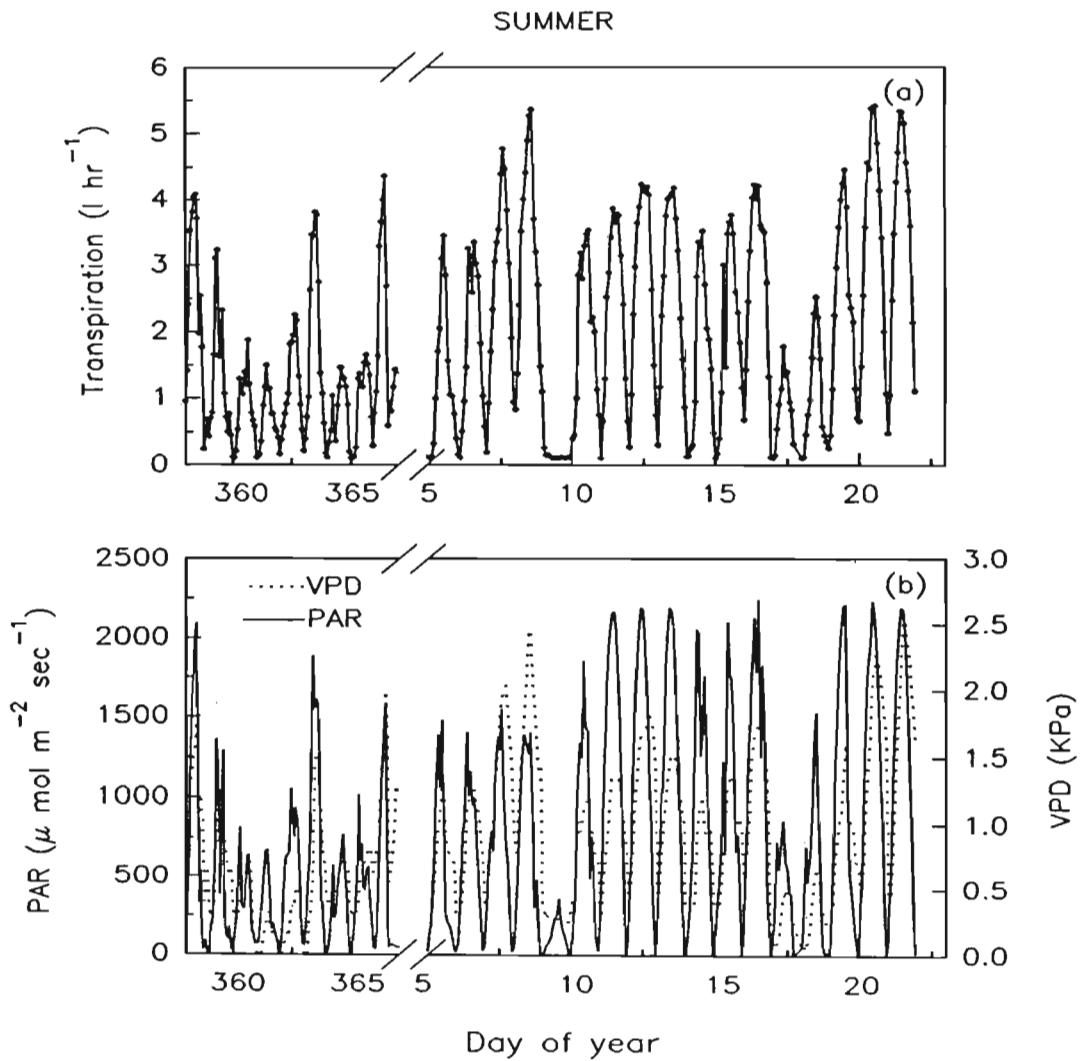
The meteorological conditions during the selected sample periods were fairly typical of those experienced during summer and autumn. The summer data set was typified by a certain amount of climatic instability (Figure 17b), with a fairly prolonged rainy spell at the start of the data set. Day-to-day variation in PAR and VPD was also significant as a result of the rainy and cloudy weather being interspersed with clear, hot sunny periods such as that observed from day 11 to 14 (Figure 17b). The highest VPD recorded was 2.6 kPa which is not considered to be particularly high. This is however typical of the Sabie area during summer (Dye *et al.*, 1990).

Figure 17 also gives some indication of the link between climatic variation and the measured transpiration rates. The rainy period between days 360 and 365 was characterised by generally low levels of insolation and evaporative demand, resulting in the low transpiration rates recorded for this period. By contrast, the dry sunny conditions experienced on days 7, 8, 20 and 21 resulted in the highest transpiration rates recorded for the period.

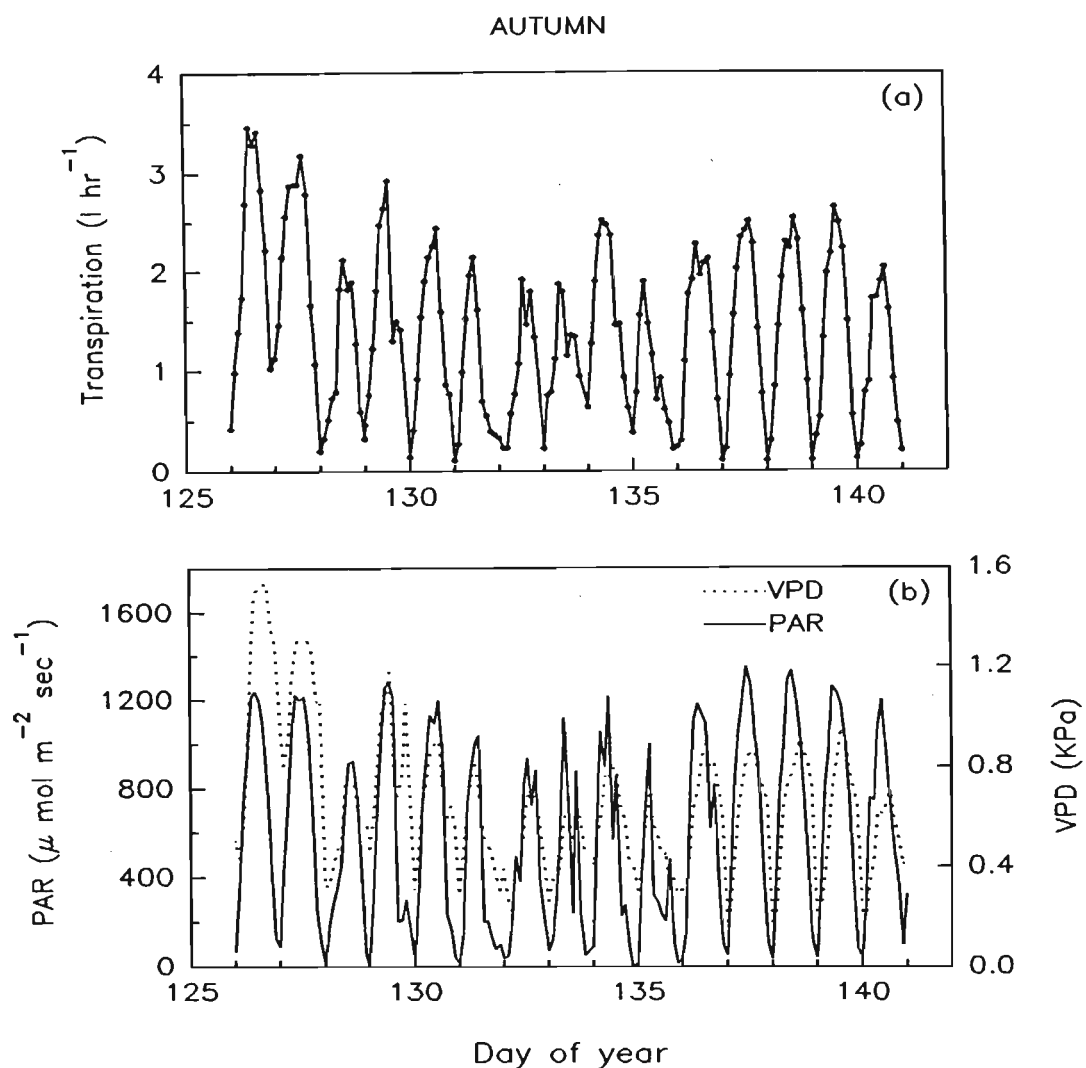
The age sequence study was conducted under clear-sky conditions to minimise climatic variation between stands. The variability observed in this data set therefore provides a rigorous test of whether the nature of the relationships observed in the age-sequence study hold true for the more variable climate normally observed.

The meteorological conditions during late autumn were slightly more stable particularly with regard to the levels of PAR experienced (Figure 18). The period was characterised by generally cloudless conditions, with a brief period of partly-cloudy weather from day

132 to day 135. In general the levels of insolation and evaporative demand were considerably lower (Figure 18b) than in summer, with a maximum VPD of 1.5 kPa. This contributed to the lower transpiration rates recorded during the autumn data set. Again, the influence of meteorology is clear, the cloudier, moister ambient conditions experienced from day 132 to day 135 (Figure 18b) resulting in the lower transpiration rates (Figure 18a) than recorded on the preceding clear, drier days.



**Figure 17.** The transpiration rates (a) recorded for the summer data set and the ambient PAR and VPD (b) recorded at the weather station for the period 24th December 1990 until 21st January 1991. The broken axis indicates three days of missing data from the weather station.



**Figure 18.** The transpiration rates (a) recorded for the autumn data set and the ambient PAR and VPD (b) recorded at the weather station for the period 3rd to the 26th of May 1991.

#### 4.3.2 Modelling the response of changes in transpiration to changes in climatic conditions.

Table 12 presents the best one, two and three factor models relating transpiration in the selected tree to the ambient climatic variables. It was evident that very few variables were required to explain a large proportion of the observed variation in transpiration. Again, VPD and PAR were the two major driving variables governing



transpiration, the best two factor models consistently having PAR and VPD terms in them. Even individually, these factors were found to account for more than 75% of the observed variation in transpiration (Table 12).

Promising regressions were found for the relationship between transpiration and the driving variables for both summer and autumn, the best two-factor models having  $R^2$  values of 0.94 and 0.95 respectively (Table 12). Initial analyses included comparisons where the necessity of logarithmic transformations of the dependent variable was investigated. This did not result in any improvement in the distribution of residuals and was therefore unnecessary (Weisberg, 1985).

*(i) Summer data*

A very mild hint of curvilinearity in the relationship between the driving variables and transpiration was evident in the introduction of either a quadratic, or a logarithmic term in the best three-factor models (Table 12). The additional variation accounted for by the inclusion of a third term in the model was however minimal and therefore not justified.

**Table 12.** Regression data for the best one, two and three factor models relating the observed transpiration in the reference tree in the water use efficiency study to the driving climatic variables in summer and autumn.

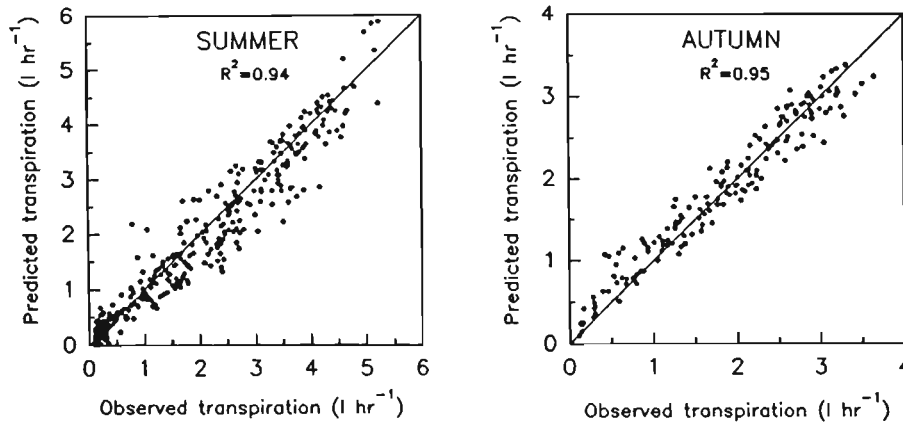
Number of terms in the model	Dependent variable	Regressors	R <sup>2</sup>
<b>SUMMER</b>			
1	Transpiration	PAR	0.79
1	Transpiration	VPD	0.75
2	Transpiration	VPD PAR	0.94
2	Transpiration	VPD Log(PAR)	0.87
3	Transpiration	PAR, VPD, PAR <sup>2</sup>	0.95
3	Transpiration	PAR, VPD, Log(PAR)	0.94
<b>Autumn</b>			
1	Transpiration	VPD	0.80
1	Transpiration	VPD <sup>2</sup>	0.77
2	Transpiration	PAR, VPD	0.95
2	Transpiration	VPD Log(PAR)	0.94
3	Transpiration	PAR, VPD, log(PAR)	0.96
3	Transpiration	PAR, VPD, PAR <sup>2</sup>	0.96

**(ii) Autumn data**

These models also returned a high R<sup>2</sup> (Table 12) and an even distribution of residuals about the mean. The best two-factor model accounted for 95% of the observed variation, while the inclusion of a third term in the model resulted in an insignificant improvement.

A comparison of the predicted transpiration rates from the best two factor model against those measured in the independent test data set revealed close agreement between observed and predicted values for both summer and autumn (Figure 19). The autumn model resulted in a relatively poor fit in extreme low-end transpiration estimates. The fit throughout the remainder of the range tested was, however, satisfactory, with a correlation between observed and predicted data of 0.95.

$$\text{Transpiration} = f(\text{PAR}, \text{VPD})$$



**Figure 19.** The relationship between the transpiration rates predicted by the best two term model and observed transpiration rates for the summer and late autumn models. An independent data set was used for these comparisons.

The best-fit regression equations are presented in Table 13. It was reassuring that the magnitude of the coefficients of the comparable regressions was consistent from summer to autumn and that the magnitude of the error term was small. The high level of significance of the models is a consequence of the large proportion of the observed variation being accounted for, and the relatively large sample size ( $n_{\text{summer}} = 360$ ,  $n_{\text{autumn}} = 182$ ). In all cases the coefficients were significant at the  $p=0.001$  level, however the intercept of the summer model was not significantly different to zero. The coefficients of the PAR and VPD terms were not significantly different ( $p = 0.05$ ) from summer to autumn. The intercepts of the two curves show a significant, negative shift from summer to autumn.

**Table 13.** The best two factor multiple regression equations relating transpiration ( $l\ m^{-2}\ leaf\ area\ hr^{-1}$ ) to the two driving environmental variables, PAR ( $\mu\ mol\ m^{-2}\ sec^{-1}$ ) and VPD (kPa). The adjusted  $R^2$  and the significance of the model are also presented.

Season	Number of terms in the model	Model	Adj. $R^2$	Prob > F
Summer	2	$3.04*10^{-5}*PAR + 4.32*10^{-2}*VPD + 0.00214$	0.924	0.0001
Autumn	2	$3.21*10^{-5}*PAR + 4.61*10^{-2}*VPD - 0.00596$	0.851	0.0001

#### 4.4 DISCUSSION

The water use efficiency data set presented the opportunity to determine whether the nature of the relationship between transpiration and the two climatic variables, PAR and VPD, varied in time.

The data from the age sequence study differed from the data used here in that hourly average VPD, PAR and transpiration data were used. This is of no real consequence because both the age sequence models and the best models in the water use efficiency data set were simple linear multiple regressions. The linear averaging of input meteorological data would therefore not influence the output from the model.

A further important difference was that the winter data for the age-sequence study were collected in September, whereas the water use efficiency data were collected during May, which corresponds to autumn. For this reason, it is not realistic to expect a seasonal shift in the water use efficiency studies data of the same magnitude as that observed in the age sequence study. Despite this, a significant seasonal shift was observed ( $p = 0.0001$ ), transpiration rates were lower in autumn than under equivalent conditions in summer.

Despite the differences cited above, the findings from this study and the age sequence study were similar. In both instances, PAR and VPD accounted for almost all of the observed variation in transpiration. Also, the nature of the relationship between

transpiration and these two driving variables was consistent with that found in the age sequence study.

Closer inspection of the regression coefficients revealed differences between the best models developed in the two data sets. The coefficient of the VPD term showed a clear trend in the age sequence data, declining with age and from summer to winter (Table 11). However, the coefficient of the PAR term is unstable showing no clear trend either with age or season (Table 11). By comparison, the coefficients of PAR and VPD showed no significant seasonal trend, but a linear shift from summer to autumn.

Although the order of magnitude of the coefficients from the single tree in the water use efficiency experiment was the same as those in the age sequence study (Table 11 and Table 13), the precise magnitude of the coefficients of the two climatic terms differed (the regressions for three-year-old trees should be used as a comparison to the regressions from the four-year-old tree studied in the water use efficiency study). This is not surprising in that the water use efficiency data set represents transpiration from a single tree and the scatter diagram presented earlier (Figure 11) illustrates considerable variation between individuals within an age class.

It is believed that the instability observed in the coefficients of PAR and VPD are a consequence of the high proportion of the variation in transpiration accounted for either PAR or VPD alone (Table 8 and Table 12). This suggests that the models may suffer collinearity problems, which could cause the observed instability in the derived models (Belsey *et al.*, 1980). However, the condition numbers and the variance decomposition proportion of the models discussed above were well within the limits beyond which they are likely to cause problems in the regression analysis (Belsey *et al.*, 1980) (The maximum condition number recorded was 10.2). None the less, with such a large proportion of the variation being accounted for by either of the two climatic variables alone, it is not surprising that the coefficients display some instability.

It was surprising that a simple linear multiple regression model would account for such a large proportion of the observed variation in transpiration. It suggests that increasing the light intensity results in an increase in the transpiration rate on a whole-canopy basis. This was counter-intuitive because studies carried out at the leaf level suggested that leaves became light-saturated, resulting in the hyperbolic relationship Dye (1987) illustrated between stomatal conductance and increasing PAR.

The observed increase in transpiration with increasing PAR is most likely due to canopy-scale processes where increasing light results in progressively greater illumination of the lower canopy leaves. It is also possibly a function of the canopy architecture of *E. grandis* when grown in plantation form. These trees develop relatively open canopies, with a leaf area index of approximately four. The leaves are often also vertically orientated. These factors would have a positive influence on the degree to which light can penetrate to lower canopy layers.

Several workers have illustrated a feed-forward response in the behaviour of stomatal conductance under increasing VPD, stomatal conductance declining with rising VPD (Dye, 1987; Cowan, 1977; Farquhar, 1978). By contrast, this work illustrated a linear increase in transpiration with increasing VPD, suggesting that, although the stomatal conductance to water vapour may decline as VPD increases, this is offset by progressively greater evaporative demand, resulting in a net increase in transpiration. This finding is conditional on the relatively low maximum VPD recorded during the two study periods. It would be interesting to establish whether the above relationship also holds true for higher VPD's such as those commonly recorded during the months of August and September where VPD's of up to 4 kPa are not too uncommon (Dye, 1987). At such a level of evaporative demand, Tyree and Sperry (1991) would suggest that this may cause catastrophic disfunctioning of the xylem, ultimately resulting in complete stomatal closure under such conditions.

Whatever the mechanism, it is clear that a simple linear multiple regression model adequately represents the relationship between transpiration and the two main environmental variables, PAR and VPD, over the range of transpiration rates observed

in the data set. This is convenient because it follows that if hourly transpiration rates are linearly related to the climatic variables, the total daily transpiration rates will also be highly correlated to linear averages or cumulative totals of these two climatic variables. The main benefit of this is that the models described in Table 13 can also be used where only daily data are available, after simple mathematical conversions to allow for the change in units.

This investigation has shown that the nature of the relationship between the two driving environmental variables and transpiration derived in the age sequence study was more broadly applicable than was expected, given that the initial relationship was developed on data with a limited range of climatic variation. Also, it confirmed that the seasonal shift observed in the age sequence data is 'real' and was not a consequence of the fact that the age sequence data were collected only on one day in each age class for each season. This study has illustrated that relationships demonstrated between transpiration and the PAR and VPD in the age sequence study are sound. The models should therefore be applicable to a broad range of meteorological data provided that the VPD does not exceed 3.0 kPa, beyond which one may experience feed-forward effects.

#### **4.5 MODEL APPLICATION TO A SAMPLE METEOROLOGICAL DATA SET**

Perhaps the most desirable addition to the work done so far would have been to perform a fully-fledged verification of the transpiration models developed for the age-sequence of *E. grandis* under conditions of non-limiting soil water availability. This would be possible if transpiration data were available for an independent age sequence of trees growing under similar conditions to those measured in this age sequence study. However, in the absence of such a data set, only two comparisons were made: firstly a comparison of the age sequence model outputs against the predictions from Dye's 1987 stomatal conductance model, and secondly a simulation exercise on a selected summer and winter data set. Neither of the above 'tests' is a

validation of the age sequence models. However, the results are presented as an appraisal of how realistic the age sequence model is.

The meteorological data were collected from an automatic weather station at Sabie Forestry Research Centre in 1988. The initial intention was to apply the age-sequence model to a longer-term data set of perhaps several years. Unfortunately, although the data were available, the quality of some of the data were questionable. For this reason I have restricted this simulation to a short period in summer and winter for which the data were of an appropriate quality. Although these data are of a more limited scope, they have been selected as representative of the seasons and therefore serve to illustrate the magnitude of the simulated differences between stands of different ages and between seasons.

One of the intentions of this exercise was to estimate the magnitude of the differences between age classes when the simplest models, where age is treated as a continuous variable, were applied. The model algorithms used are presented in Table 14.

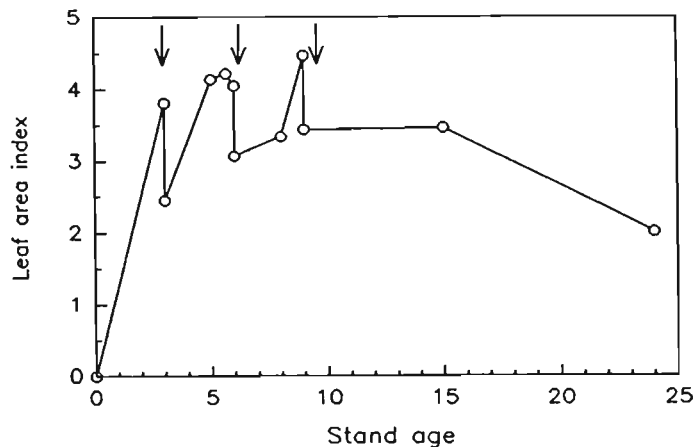
**Table 14.** The model algorithms used to predict transpiration ( $\text{mm hr}^{-1}$ ) from stand age (years), VPD (Kpa) and PAR ( $\mu \text{ mol m}^{-2} \text{ sec}^{-1}$ ). Essentially, the difference between Model 1 and Model 2 below, is that the logarithm of PAR is used in Model 2.

Season	Model algorithm	R <sup>2</sup>
MODEL 1 Summer	Transpiration = $\text{LAI} * (-5.81 * 10^{-3} * \text{Age} + 6.62 * 10^{-2} * \text{VPD} + 5.82 * 10^{-5} * \text{PAR} + 0.0626)$	0.73
Winter	Transpiration = $\text{LAI} * (-5.54 * 10^{-3} * \text{Age} + 2.46 * 10^{-2} * \text{VPD} + 5.72 * 10^{-5} * \text{PAR} + 0.0573)$	0.72
MODEL 2 Summer	Transpiration = $\text{LAI} * (-6.66 * 10^{-3} * \text{Age} + 7.68 * 10^{-2} * \text{VPD} + 1.58 * 10^{-2} * \text{Log}(\text{PAR}) + 0.0161)$	0.70
Winter	Transpiration = $\text{LAI} * (-5.91 * 10^{-3} * \text{Age} + 2.95 * 10^{-2} * \text{VPD} + 1.40 * 10^{-2} * \text{Log}(\text{PAR}) + 0.0157)$	0.64

The models developed in the age sequence study predicted transpiration per unit leaf area for a given set of climatic conditions. A simulation of the transpiration per unit ground area or 'rainfall equivalent' requires information on the variation of the leaf area index (LAI) with age. Data from an unpublished study are presented in Figure 20 and represent a collation of all the reliable leaf area index data for *E. grandis* from the Sabie area. As such, only those data collected via destructive sampling were included



due to uncertainties regarding the accuracy of leaf area index-measuring instruments at the time the work was done. These data were used to estimate 'typical' leaf area indices of *E. grandis* stands with age.



**Figure 20.** The change in the leaf area index of *E. grandis* stands sampled in the Sabie area of the Eastern Transvaal. The arrows indicate the timing of management thinnings on *E. grandis* trees grown on a saw-log rotation (Data reproduced with the permission of P.J. Dye).

The regular management thinnings reduce the over-story LAI by about one, maintaining peak LAI's at about four. Data from an un-thinned stand indicates that the LAI would rise to about 5.8 if the stands were not thinned.

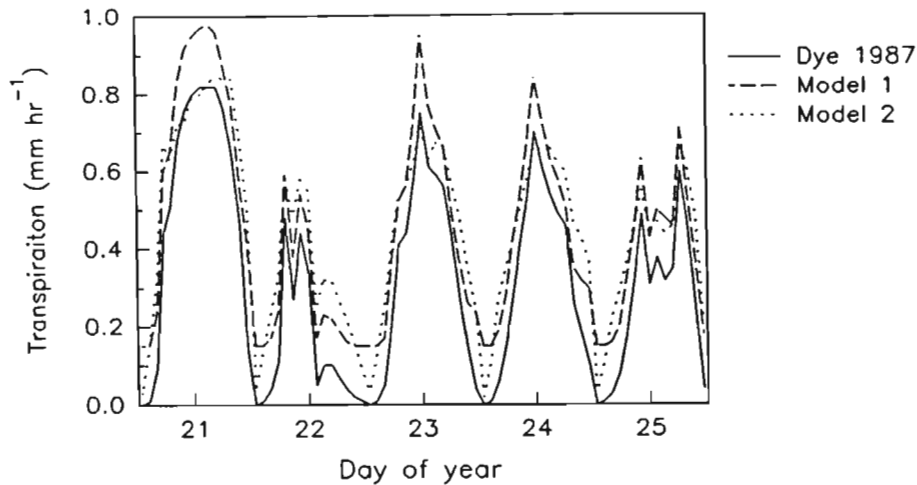
The simulation exercise also required an estimation of when to switch from the application of the 'summer' models to the 'winter' models. Ideally, this should be based on experimental data. The only data available were the stomatal conductance data collected by Dye (1987) for the single six-year-old *E. grandis* tree at Frankfort State Forest. The seasonal changes occur in mid-September and at the end of April (Dye and Olbrich, 1993). For the purposes of the simulation, it was decided to consider the period from the beginning of October to the end of April as summer, while, winter was considered to be from the beginning of May to the end of September. The sharp rise in transpiration at the end of September, indicating the end

of the winter period, appears clear on year-long transpiration data such as that in Dye (1993). However, the transition from summer to winter is not as clear and appears to occur gradually. The data presented in the figures are an extract of the above periods selected to demonstrate key features of the simulation exercise.

#### **4.5.1 Predictions of hourly transpiration.**

The figure illustrating a comparison of the model outputs of three models run in summer conditions (Figure 21) illustrates considerable agreement between the three models. Dye's (1987) model was formulated on stomatal conductance data from several canopy layers in a single six-year-old tree and the output run through the Penman Monteith equation to derive an estimation of transpiration. The way in which the three curves track each other through the course of the day despite considerable meteorologically-induced variation in transpiration is particularly encouraging.

Peak values from Dye's model were consistently lower than those predicted using the age sequence models. The model in which the logarithm of PAR is used presents a more conservative estimate of peak transpiration (Figure 21), and on clear days, such as day 21, the peak is predicted as occurring later in the day. Model 2 predicts higher transpiration rates than the other models during the mid-morning and mid-afternoon periods.



**Figure 21.** Hourly transpiration rates simulated by three models for a six-year-old stand of *E. grandis* for a period of five days during summer. Model 1 and Model 2 were the best models developed from the age sequence study data. Both models were of the form  $Transpiration = f(Age, PAR, VPD)$ , except that the logarithm of PAR was used in Model 2. The data have been edited to show the predicted transpiration rates from 5h00 till 19h00 and the tick mark corresponds to 12h00.

Model 1 predicts relatively high transpiration rates in the early morning and late evening, co-inciding to conditions of low PAR. This is a mathematical consequence of the way the model was set up. If PAR is zero, only the PAR term returns a zero value, the VPD and age term still have an effect. This is also a consequence of applying the model to data beyond the limits on which it was developed - the data on which the model was developed included only daytime data. This is taken care of in the model which uses the logarithm of PAR because this model predicts more realistic values for the early morning and late evening period.

Day 22 exposes a further interesting feature. The discrepancy between the two models developed on the age sequence data and that developed by Dye (1987) is greatest under heavily overcast conditions (Figure 21). Both Model 1 and Model 2 predicted higher transpiration rates than Dye's model, particularly for the afternoon period. Dye acknowledged these conditions as being problematic and commented that this was probably due to the differences in stomatal conductance as one moved down through the canopy. Ultimately, this problem resulted from the data used to develop the model

being based on sub-samples of the canopy, whereas these issues are avoided if whole-tree estimates can be obtained.

Notwithstanding the differences emphasised above, given the good agreement between the models over the course of the day, it would be surprising if the predicted total daily transpiration estimates differed considerably.

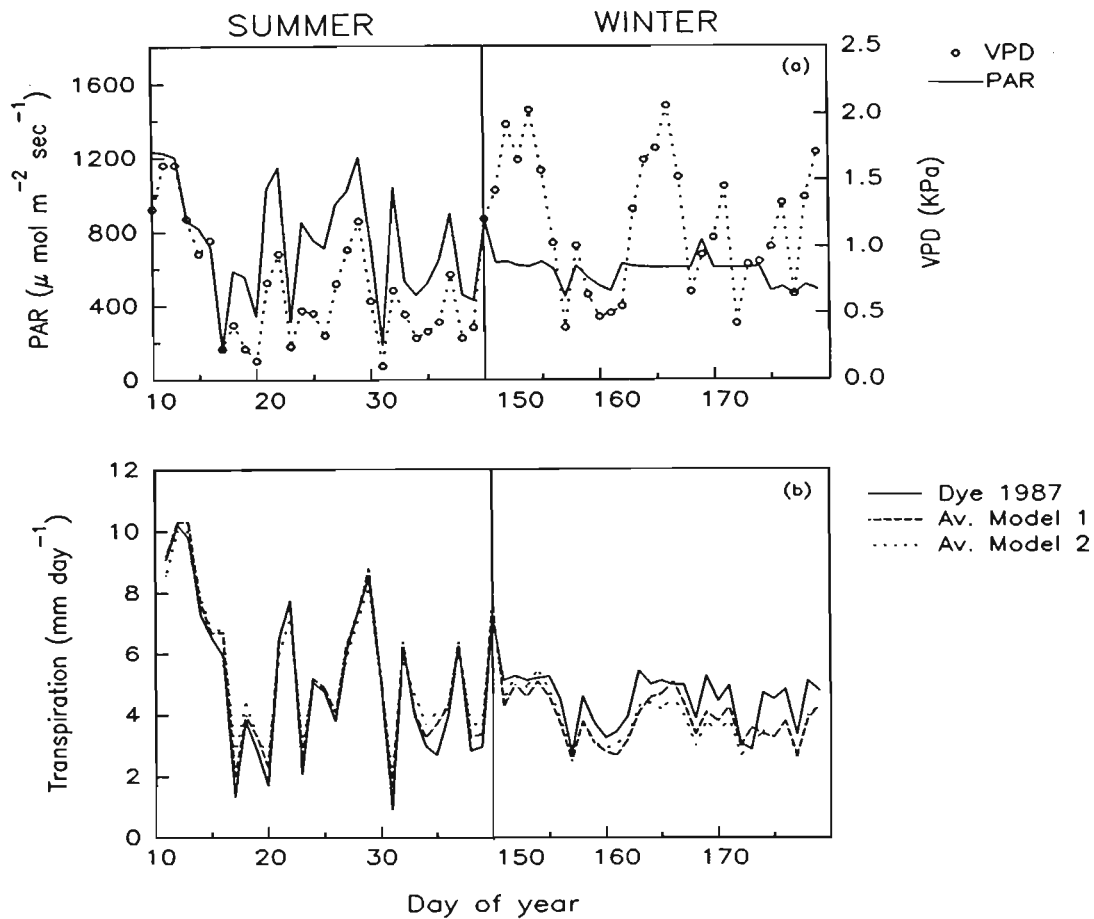
#### **4.5.2 Total daily transpiration**

The simulation was conducted by running the models on hourly-mean meteorological data. This resulted in predictions of hourly transpiration. These hourly values were totalled over the course of a day (from 05h00 to 19h00) to derive estimates of total daily transpiration in mm per day.

The mean daily meteorological conditions for the selected summer and winter periods were quite contrasting (Figure 22a). The summer conditions were characterised by considerable instability with intermittent periods of heavy clouds which, in turn, resulted in the dramatic fluctuations in the mean levels of PAR and VPD over the 30 days' data presented. By contrast, the winter conditions were more stable, with very little day-to-day variation in PAR. These clear-sky winter conditions are typical of the Sabie winter period. The levels of VPD did fluctuate fairly dramatically during winter, reaching a maximum hourly-mean value of 2.5 kPa.

The predictions by the three models followed a remarkably close pattern, particularly during the summer period (Figure 22b). It is co-incidental that there is such a good match between the model output from Dye's transpiration model and the two age sequence models because the data for the age sequence models represent the mean prediction for the five age classes simulated. The agreement between the models over the winter period was not as good as was the case in summer and possibly relates to the difficulties experienced with Dye's model under the lower PAR levels

experienced during the winter months. Essentially, the two age-sequence models give very similar predictions over the course of the sample data set presented.



**Figure 22.** The daily average VPD and PAR (a) in the selected sample periods representing summer and winter. The predicted variation in transpiration from three models, Dye's stomatal conductance model, and the best two models developed using the age sequence data is presented in (b). Model 1 was of the form  $Transpiration = f(\text{age}, PAR, VPD)$ , while the logarithm of PAR was used in model 2. The output of Model 1 and Model 2 are the mean of the five age classes simulated.

The mean daily transpiration rates predicted by the model where age was treated as a continuous variable was generally higher than that predicted by the models for each of the age-classes individually (Table 15) for the summer data. The situation in winter was a little different, with no clear trend in the data.

The peak transpiration rates predicted by the three models applied in Figure 22 are relatively high by comparison to those published elsewhere in the literature. The

highest rate reported in the literature was 7.35 mm per day for nine, three-year-old *Eucalyptus tereticornis* trees over a period of ten days in India (Calder, 1992). This value is similar to that estimated for the three-year-old trees during summer, and larger than the daily average transpiration estimates for the remaining age classes cited in Table 15. Penman-Monteith hourly estimates of transpiration indicate considerable day-to-day variation in transpiration at Calder's (1992) site, suggesting that the peak transpiration rates could well have been considerably higher than the mean he cited. It was also interesting to note such close agreement between the age sequence models' predictions and those from Dye's stomatal conductance model. These two pieces of corroborative evidence suggest that although the transpiration estimates predicted above are relatively high, eucalypts may well have the potential of transpiring at such high rates, particularly when young.

**Table 15.** Mean daily total transpiration estimates simulated for five age classes of *E. grandis*. Two models developed in the age sequence study are applied: Model 1, which treats age as a continuous variable, and Model 3, in which each age class is treated independently (Table 11). These data represent averages over the thirty-day period selected for summer and winter illustrated in Figure 22.

Season	Model	Mean daily transpiration $\pm$ sd (mm day <sup>-1</sup> )					
		Stand age (years)					Mean
		3	6	8	13	16	
Summer	Model 1	7.7 $\pm$ 2.5	7.4 $\pm$ 2.8	5.4 $\pm$ 2.3	4.0 $\pm$ 2.3	3.2 $\pm$ 2.1	5.5 $\pm$ 2.4
Summer	Model 3	7.6 $\pm$ 3.9	5.8 $\pm$ 3.2	4.5 $\pm$ 2.3	5.3 $\pm$ 2.3	2.9 $\pm$ 1.5	5.2 $\pm$ 2.6
Winter	Model 1	5.9 $\pm$ 0.8	5.4 $\pm$ 0.9	3.9 $\pm$ 0.7	2.5 $\pm$ 0.7	1.7 $\pm$ 0.5	3.9 $\pm$ 0.7
Winter	Model 3	5.0 $\pm$ 1.6	6.8 $\pm$ 1.4	3.7 $\pm$ 0.8	2.3 $\pm$ 0.5	2.7 $\pm$ 0.3	4.1 $\pm$ 0.9

The application of the models to the sample data still bears scrutiny. There are several possible explanations for the high predictions. Firstly, in the application of models such as those applied in this simulation exercise, it is almost inevitable that the models will be applied to data beyond the range on which they were developed. In the case presented above, Dye's stomatal conductance model was developed on summer-time data with a peak VPD of 1.9 kPa, while the age-sequence data was developed on data

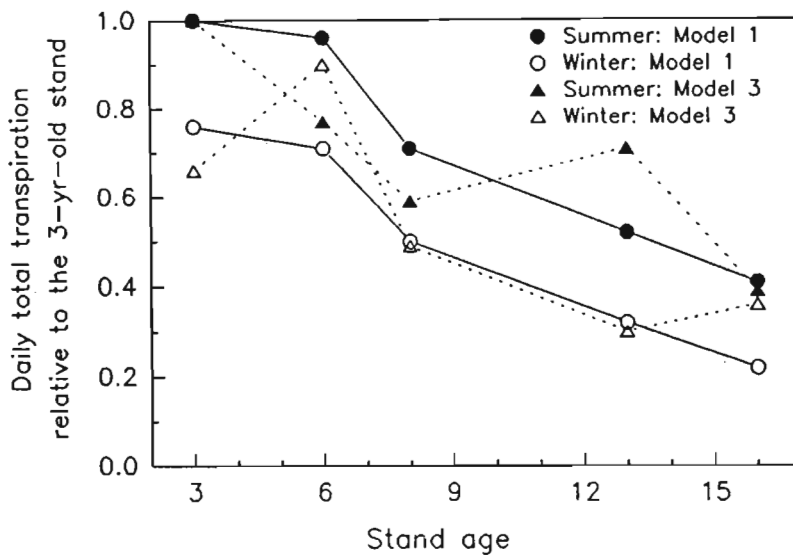
with a peak VPD of 2.5 kPa. In the simulation exercise, the VPD exceeded 2.0 kPa for between 20 and 40% of the data for days one to three in Figure 22, and reached a peak of 2.8 kPa on day 3. While this is beyond the range of the data on which Dye's model was developed, nearly all the values fall within the range of VPD's of the data set on which the age sequence models were developed. Values predicted for the remainder of the period were for meteorological situations falling within the limits of the data sets on which the models were developed.

A further issue that deserves consideration is that the LAI used in the model algorithms (Table 14) was derived from the LAI curve presented in Figure 20. In order to be consistent, the pre-thinning values were used in all cases. It is clear that if the post-thinning values had been used, it would have effected a proportional decrease of about 25% in the transpiration estimate because LAI is linearly related to transpiration in the model algorithm. Indeed, the actual LAI of the stands sampled may well have been lower than that used in the simulation exercise. It is also not clear to what extent it is valid to use LAI as a simple linear multiplier parameter. Establishing to what extent the relationship between transpiration per unit leaf area and the two driving environmental variables changes with a change in LAI requires further attention. However, it is unlikely that the LAI of the stands sampled in the age sequence study differed dramatically from those in the LAI survey, given the criteria on which the age sequence stands were selected and the meteorological conditions during the study.

The results of the above simulations were summarised to examine the net effect of declining transpiration per unit leaf area with age, the changes in LAI as *E. grandis* stands age, and the decrease in transpiration with season. The average daily transpiration predicted in the simulation models was scaled relative to that predicted for the thirty day summer period for the three-year age class (Figure 23). This was done to present a summary of the combined effect of the three main trends identified.

Figure 23 illustrates several features quite clearly. Firstly, no matter which models are used, the younger trees transpire at faster rates than the older trees (Figure 23). Predictions of transpiration are sensitive to the choice of model. Model 1 would be the

more appropriate choice to examine the nature of the differences between stands of different ages because this is a long-term simulation exercise. The simulations predict that the transpiration from a sixteen-year-old stand is only about 40% of that of a three-year-old stand under the same conditions in summer (Figure 23). The relative difference in transpiration between the three-year-old stand in summer and a sixteen-year-old stand in winter is even greater, with the three-year-old stand transpiring almost 5 times as much as the sixteen-year-old stand.



**Figure 23.** The simulated average daily total transpiration estimated for a range of age classes, expressed as a proportion of that estimated for the three-year-old stand of *E. grandis* in summer. The simulation was conducted using Model 1, in which age is treated as a continuous variable and, Model 3, which models each of the age classes individually.

The seasonal effect is also clear in the output of the simulations. The output of Model 1 (Figure 23) illustrates this effect in the 25% decline in transpiration from the three-year-old stand from summer to winter. This decline increases with tree age because Model 1's curves run parallel to one another, resulting in a 50% difference between summer and winter transpiration from the sixteen-year-old stand. These simulation results are not independent of meteorology, as the data set used was representative of a period in summer and a period in winter. The differences between the summer



and the winter curves (Figure 23) therefore represent the combined effect of (i) the change due to the different meteorological conditions experienced in the seasons and (ii) differences due to the seasonal shift in the response function.

The figure also indicates that Model 1 would be the most appropriate model to use in order to compare different silvicultural practices such as rotations. This model is less sensitive to the possible anomalies of the original stands used to derive the model eg. the six-year-old stand in winter, which is evident in the application of Model 3. Hence, by including age as a variable in the model, individual differences in a particular age class are minimized, so that the overall trends become clearer.

There is, however, a danger in the application of Model 1 to age-classes beyond those on which it was developed. Given the decline in transpiration with age, represented by the negative coefficient of the age term in the model, if the model were applied to trees old enough it would predict negative transpiration rates! A similar problem exists in reverse if the model were applied to trees younger than three-years-old. Further data are required to define the curve beyond the sixteen-year-old age class and the development to the three-year-old stage.

One limitation of the regression model developed in this thesis is that it can not reasonably be applied on data beyond the range of the data on which the models were developed. For example, the application of the model should be restricted to daytime data only. In the model algorithm predicting transpiration, PAR and VPD are additive terms (Table 11). One consequence of this is that if PAR is set to zero, as would be the case at night time, the algorithm would predict transpiration as a function of the level of VPD alone. We have no indication to suggest that night-time transpiration occurs in eucalypts and the application of the model to night time data should be avoided.

The modelling exercise presented here addresses only one of at least four hydrological loss components. The transpiration models presented in this thesis represent one part of a bottom-up modelling strategy to predict the water losses from eucalypt

plantations. A model has also been developed to predict water loss through the process of canopy interception (Dye, 1993b) and work is currently underway to develop a model to predict litter interception and evaporation. Indications are that interception losses are minor by comparison to the loss of water through the process of transpiration.

The final assessment of the hydrological impact afforestation with eucalypts will have to incorporate models covering all the hydrological components. It is possible that changes in one component may be offset by simultaneous changes in one of the other components which could have an important bearing on the net outcome. For example, although transpiration may well decline with age, interception may increase with age. This is possible because although the LAI remains relatively stable, the older trees are taller, resulting in better ventilation and potentially elevated evaporation rates from the canopy. Also, as the stands age the total stem surface area increases, contributing to the interception losses.

The simulations presented here are also subject to the nature of the meteorological data used. Because a simple linear regression model was developed, the relative differences between stands should remain constant irrespective of the input meteorological data (provided the same LAI data are used). However, the absolute predictions will change given different input data. The utility of the age sequence model is that it allows predictions under all weather conditions, including rain. This was illustrated in the transpiration rates derived from the water use efficiency study.

This chapter has served to confirm that the nature of the relationship between PAR and VPD, and transpiration can be captured in a simple, linear multiple regression model. This was consistent with that developed in the age sequence study (Chapter 3) on only one day's data from each of five stands in summer and winter. The relationship appears stable in time, merely showing a change with season. The peak absolute rates of transpiration predicted were rather high, particularly for young stands in summer, and still require verification on an independent data set. However, it was encouraging that there was a remarkably close agreement in the behaviour of Dye's

model, developed on stomatal conductance data, and the age-sequence data. Also, Calder's (1992) results indicated that under certain circumstances Eucalypts can achieve quite considerable transpiration rates.

## CHAPTER 5.

# EXPLORING HPV SAMPLING STRATEGIES FOR MEASURING TRANSPIRATION FROM INDIVIDUAL TREES AND STANDS OF FOREST TREES

### 5.1 INTRODUCTION

One of the objectives of the hydrology programme at the Division of Forest Science and Technology at the CSIR (Council for Scientific and Industrial Research) in South Africa is to develop models to predict the hydrological impact of forest plantations. The focus of the age sequence study (Chapter 3) was the prediction of transpiration from plantation trees of varying age. This showed that, under the same conditions of evaporative demand, transpiration rates of *E. grandis* were lower in winter than in summer, and lower in the older trees than in the younger ones. Also, the results showed considerable variability between trees within a stand. This was not related to tree size or water availability, and is possibly genetically determined. A further source of variability may also be due to a sampling error in the heat pulse velocity procedure. Whatever the source of this variability, the implication is that either more trees need be sampled to accurately estimate the mean transpiration from a forest stand, or perhaps the limited probes need to be implanted in a different arrangement.

If this study were repeated, one option would be to purchase sufficient data loggers and probes to sample ten or twelve trees simultaneously within a stand. However, resources are often limited and the cost of adopting this approach would limit its application.

A second option is to explore the possibility of altering the probe allocation strategy. For example, it may be possible to use the predictable way in which sap flux density varies with depth in the sapwood to reduce the required sampling intensity per tree (number of probes per tree). This would increase the number of trees that could be sampled within a stand. This would also improve the estimate of stand transpiration, perhaps at the cost of decreased resolution on the individual. Yet another possibility is to allocate one probe to a random depth in each of several trees in a stand.

To explore different probe allocation strategies, three components must be investigated:

- i) The variation in the sap flux density within the sapwood of individual trees,
- ii) the variation in profiles between trees within a stand, and
- iii) the variation as trees grow older and the total sapwood area increases.

The issue of how many probes should be used per tree was addressed qualitatively in the verification study. The results indicated that four probes per tree were adequate for three year old trees, while it was felt that at least eight probes should be used in trees larger than about 20 cm in diameter.

If this question is examined further, through collection of additional sap flux density profiles, it will be possible to determine the sampling needed to obtain reliable estimates of an individual tree's transpiration rate (i.e. within a given percentage of the mean). This in itself is important for the conventional use of the instrument. Dye *et al.* (1991) have done this for *Pinus patula*, and showed that twelve probes per tree resulted in 76.5% of the samples lying within 10% of the true value.

The objectives of this chapter are to address the questions:

- i) How many probes are required to accurately estimate transpiration from both individual trees and in a stand of trees?
- ii) What strategy should be used to determine to what depth the probes should be implanted in the selected sample trees?.

## **5.2 METHODS**

### **5.2.1 Study site**

The selected study site was Frankfort State Forest near Sabie in the Eastern Transvaal province of South Africa. This was the site where all other heat pulse work reported on in this thesis was conducted. It was important that this research could be used to interpret the results achieved in the other chapters, without introducing unnecessary variation due to site.

### **5.2.2 Field data collection**

Two stands of *E. grandis* were selected for this analysis. The first, compartment A19, was planted to three-year-old trees which had not yet been thinned. The second, compartment A15, was an eight-year-old stand which had undergone two regular management thinnings. Ten trees were randomly selected for analysis in each stand. However, two of the HPV loggers malfunctioned on each occasion, reducing the total number of trees in each age class to eight.

Probe implantation holes were drilled and probes installed in each of the selected sample trees 14 days prior to the study, to allow adequate expression of the wounding reaction. The holes were drilled to a total depth of 50 mm to ensure that they passed through the sapwood into heartwood in each of the selected trees. To account for the

differences in the sapwood area between the smaller three-year-old trees and the larger, eight-year-old trees, four sets of probes were implanted in the three-year-old trees and eight sets of probes were implanted in the eight-year-olds. This resulted in a similar sampling intensity (probes per unit sapwood area) for the two selected age classes (Table 16).

The strategy adopted was to take successive measurements, at intervals of approximately 15 minutes, along each radius, by withdrawing the thermistor probes by increments of 3 mm at a time. All HPV measurements at any particular depth were made at the same time. The initial depth of insertion in both age classes was 40 mm beneath the cambium. Measurements in the three-year-old age class were made between 12h00 and 14h30 on the 12 May 1993, while the measurements in the eight-year-old trees were made between 11h50 and 14h30 on the 7th of June 1993. Cloudless conditions were selected for this study to minimise variation in sap flow rates due to changes in environmental conditions over the period of sampling. In addition, the depth of one probe was held constant in each age class, to monitor any changes in sap flow rate over the course of the study.

At the end of the study each of the sample trees were felled and a disk containing the probe implantation holes removed for further analysis. The sapwood thickness was determined using the standard methyl orange staining technique (Bamber and Fukazawa, 1985). The sapwood area was calculated on each disk by subtracting the area of heartwood from the total area of the disk. Measurements were also made of the sapwood thickness along each of the radii.

The sections of the disk containing the holes were excised using a sharp chisel and the exact separation of the probes measured at the cambium and at a depth 40 mm beneath the cambium. The separation of the probes at each of the depths was then calculated by linear interpolation. The wound size was measured on four positions along each radius and the mean calculated for each radius.

These data were analyzed in the conventional manner to determine sap flux densities (SFD) corresponding to the measurements made at each depth along each of the radii. The mean SFD was calculated for the reference probe in each age class. The deviation of measurements made by the reference probe at each interval from the mean reading was used as a correction factor. SFD's recorded by each of the other probes at that time were then adjusted using the correction factor. Sap flow rates were found to be stable in the eight-year-old trees, but did vary in the three-year-olds.

### **5.2.3 Data analysis**

The aim of the study was to evaluate how many probes are required to estimate sap flow accurately in both the individual trees and also for the stand. A combination of random and non-random probe implantation strategies were compared:

#### **(i) Individual trees**

There were two facets to this problem:

- a) How many probes should be used to estimate transpiration accurately in individual trees?
- b) What strategy should be used to decide on the depth to which each probe is implanted?

These questions were addressed using a Monte Carlo-style analysis in repeatedly selecting independent samples at random from the population of SFD data points collected for each tree. In this way it was possible to simulate sampling the population of SFD values 300 independent times to build a bootstrapped data set (Efron and Tibshirani, 1986). Care was taken to simulate the sampling as realistically as possible. For this reason, if only four samples were selected from each tree, these would be drawn from separate radii. In cases where more than one sample was selected from each radius, the random samples were selected without replacement, so that each



sample selected was unique. In effect, this realistically simulated the situation where multiple thermistors are implanted in each probe.

Both random and stratified probe implantation strategies were simulated. These procedures were used to select SFD data for each tree. The sap flow estimated from each independently selected data set was calculated by multiplying the mean SFD for each tree by the sapwood area, and dividing by 1000 to convert the units to  $\text{L hr}^{-1}$ . The mean sap flow predicted in each of the simulations was calculated for each of the trees, and the proportion of the data points within ten and fifteen percent of the population mean calculated.

The following specific conditions were simulated:

- a) Random sampling: One to sixteen probes per tree, each placed at a randomly selected depth within the sapwood.
- b) Stratified, non-random sampling: four or eight probes each implanted to a specific depth within the sapwood (the conventional approach).
- c) Non-random sampling: Four or eight probes all implanted to the same depth beneath the cambium.
- d) Four or eight probes all implanted to a specific depth representing a proportion of the available sapwood thickness.

**(ii) *Sampling stands of trees***

The approach was to realistically simulate a situation researchers may face when deciding on sampling transpiration from a relatively homogeneous stand of trees. Forestek's heat pulse loggers are designed to accommodate a maximum of twelve probes, each of which can be used independently. My early heat pulse research was conducted with two 'Custom' heat pulse loggers which could accommodate a total of eight probes. This simulation examines a situation where a researcher has either three 'Custom' loggers, or one Forestek logger, with a total of twelve probes at his disposal. The question being addressed here is that, given the limited equipment available, how

should it be used to estimate the transpiration of a **stand of trees**? Again, the question of how they should be used has two parts:

- a) How many trees need be sampled within the stand?
- b) To what depth should the probes be implanted within the sapwood of the selected trees?

A prospective researcher could contemplate implanting all twelve probes in a single tree, or at the other extreme, one probe in each of twelve trees in the stand. These probes could be implanted at random depths within the sapwood of the sample trees, or, a stratified procedure could be used. Combinations of these two procedures could also be applied. All the possible permutations of using 12 probes in a stand, ranging from two probes in each of six trees, to 12 probes in only one tree, were tested. In addition, the option of using only one probe per tree in each of the eight sample trees was also tested. However, this strategy is equivalent to the use of only eight probes in the stand.

There may be advantages to implanting probes in a stratified, rather than random fashion, within the sapwood. In an extension of this to the stand situation, the following probe depth situations were simulated:

- a) Probes implanted to a random depth within the sapwood.
- b) Probes implanted to a random depth within a restricted range centred about the depth at which average sapflow rates were recorded (Figure 28 a and c).
- c) Probes implanted in a stratified fashion: the sapwood of each sample tree was divided into four concentric annuli of equal area, and one reading selected midway through the annulus. Simulations were conducted in such a way as to ensure that each of the annuli was equally represented. Where replicate readings were selected from trees these were always selected from different annuli.

The method used was to conduct a Monte Carlo-style analysis where random samples were drawn repeatedly from the same population of data points which represent the 'true stand transpiration'. The procedure outlined in Efron and Tibshirani (1986) was followed to randomly select one thousand 'bootstrap' samples. These randomly selected data sets were then used to estimate the stand transpiration rate, and the deviation from the 'true value' was calculated. Two statistics were calculated: firstly, the deviation of the mean transpiration estimate for each simulation from the 'true estimate'. This reflects the degree to which the particular strategy introduces 'bias' ie. over- or under-estimates of the stand transpiration. Secondly, the standard error of the estimates, which represents the precision of the estimate. This describes the variation about the mean stand transpiration estimated in the simulation. A large standard error would imply that, were the particular strategy implemented in the field, there would be a relatively high risk that the estimate derived may differ considerably from the true stand transpiration.

The bootstrap samples were used to calculate stand transpiration in two ways:

*a) Un-weighted stand transpiration*

The unweighted stand transpiration was calculated by averaging the randomly selected readings from each of the twelve probes and multiplying by the total sapwood area of the stand. I have referred to this as the unweighted estimate because the method does not give readings taken from larger trees more influence on the transpiration estimate than those from smaller trees.

*ii) Weighted stand transpiration*

The weighted stand transpiration was calculated by multiplying the average sap flux density for each tree by the sapwood area of that tree. The transpiration rates of the sample trees were then totalled. A scaling factor was then calculated by dividing the total sapwood area of the 'stand' by the total sapwood area of the sample trees. I have called this a weighted analysis because larger trees are given relatively more weight by virtue of their larger sapwood area.

*(iii) Assessing the accuracy of the strategy used to sample transpiration in the age-sequence study*

In the age-sequence study, four probes were implanted in each of the selected sample trees in the three-year-old age class, and eight probes per tree in all the older age classes sampled. In all cases, except the thirteen-year-old stand, five trees were sampled per stand. Probes were implanted to stratified depths within the sapwood in the selected trees. The data collected from the sampling optimisation study were used to simulate the situation in the age sequence study. This was to provide an indication of the accuracy of the estimates gained in the age-sequence study.

## **5.3 RESULTS**

### **5.3.1 Tree physical characteristics**

In both stands there was considerable variation in the size of the randomly selected trees. The diameter at breast height of the largest three-year-old tree was 32% larger than that of the smallest tree sampled, while the largest eight-year-old tree had twice the diameter of the smallest (Table 16). This range is typical of trees grown from seed, whereas greater uniformity is achieved in clonal stands.

The sapwood of the three-year-old trees sampled was also unusually thick, four of the trees having a sapwood more than 5 cm thick. Also, the distinction between sapwood and heartwood was not as clear as previously experienced, deeper parts of the sapwood tending to stain a mid-orange colour. This suggested that the deeper parts of the sapwood were in the process of becoming non-functional, but that tylosis deposition had not yet progressed to the stage where it causes a deep red stain in reaction with methyl orange.

The eight-year-old trees sampled were considerably larger than the three-year-old trees, having a mean diameter twice that of the three-year-olds. The sapwood of the eight-year-old trees was, however, only about half as thick as that measured in the

younger three-year-old trees. The total sapwood area of the older trees was still considerably larger than the sapwood area of the younger trees (Table 16).

A total of eight radii were sampled in the older trees, while only four radii were sampled in the younger trees. This was to ensure an approximately equal sampling intensity between the two age classes. However, unexpected differences in the sapwood thickness resulted in the three-year-old trees being sampled slightly less intensively than the eight-year-old trees.

The wound size of the three-year-old trees was found to be fairly consistent between trees, the standard deviation being marginally more than 10% of the mean recorded for the ten sample trees. The wound sizes recorded in the eight-year-old trees were even more stable, with substantially less variation between samples (Table 16).

### **5.3.2 Sap flux densities recorded in the sample trees**

The mean sap flux density (SFD) recorded in the three-year-old trees was  $62.1 \text{ cm}^3 \text{ cm}^{-2} \text{ hr}^{-1}$ , and ranged from a maximum of 77.5 to a minimum of  $46.3 \text{ cm}^3 \text{ cm}^{-2} \text{ hr}^{-1}$  (Table 17). The maximum SFD recorded for each of the sample trees was considerably more variable, ranging from 241.9 to 115.0. By contrast, the eight-year-old trees were found to have lower SFD's, tree number four having a mean sap flux density of only 16.6 and a maximum of  $33.1 \text{ cm}^3 \text{ cm}^{-2} \text{ hr}^{-1}$ . It is, however, difficult to draw any direct conclusions from the comparison between age classes because the data were collected on different days. The apparent differences may be due to differing climatic conditions on the days on which the two age classes were sampled.

The sap flux density profile data (Figure 24) show that all sixteen trees sampled had similar profiles bar tree 3 in the three-year-old age class and tree 4 in the eight-year-old age class. Sap flux densities were generally highest near the cambium, peaking at a depth approximately 12 mm beneath the cambium. Sap flux densities showed a general decline as the heartwood was approached. Tree three in the three-year-old age class was unusual in that the peak SFD was recorded at a depth of 27 mm, and

the SFD's recorded at depths greater than 30 mm were considerably higher than those recorded in the other trees.

**Table 16.** The diameter at breast height, sapwood thickness, sapwood area, wound size and sampling intensity of the eight trees selected for analysis in each of the three- and eight-year-old age classes.

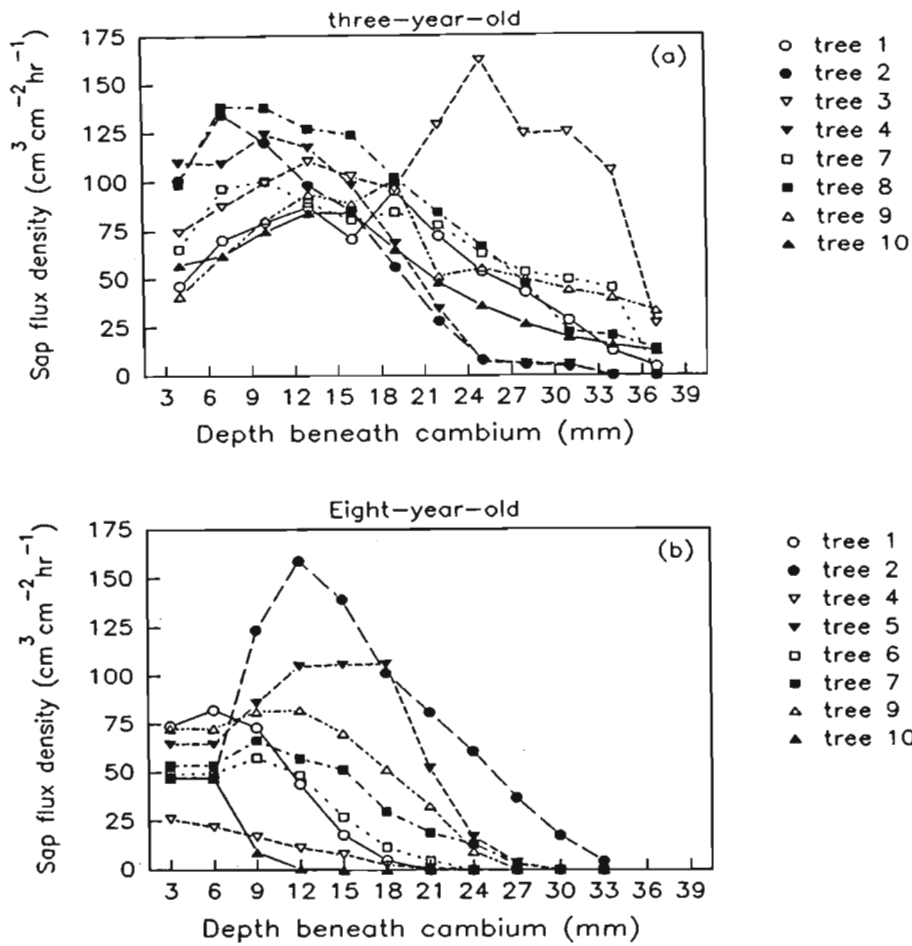
Age	Tree	Diameter at 1.3 m (cm)	Sapwood thickness (cm)	Sapwood area (cm <sup>2</sup> )	Wound width (mm)	Sampling intensity (cm <sup>2</sup> <sub>sapwood</sub> /probe)
3	1	11.0	4.0	86.9	2.2	21.7
	2	9.5	3.1	61.3	3.0	15.3
	3	11.7	5.8	107.5	2.9	26.9
	4	10.1	3.2	68.6	3.0	17.1
	7	14.0	6.6	152.4	2.5	38.1
	8	10.7	5.3	89.1	3.0	22.3
	9	13.1	4.6	123.1	2.5	30.8
	10	10.0	5.0	77.8	2.5	19.4
<b>Mean ± sd</b>		11.23 ± 1.49	4.68 ± 1.16	95.83 ± 28.44	2.70 ± 0.31	23.96 ± 7.11
8	1	20.4	2.1	118.5	3.0	14.8
	2	25.9	3.1	223.1	3.0	27.9
	4	17.1	2.0	94.6	3.0	11.8
	5	28.3	2.5	202.6	3.2	25.3
	6	22.6	2.1	133.5	2.9	16.7
	7	25.0	2.6	179.8	3.0	22.5
	9	24.9	2.5	175.1	3.2	21.9
	10	14.0	1.5	57.6	3.0	7.2
<b>Mean ± sd</b>		22.25 ± 4.51	2.28 ± 0.46	148.11 ± 53.01	3.02 ± 0.09	18.51 ± 6.63

As expected from the sapwood thickness measurements, sap flow was often recorded at greater depth beneath the cambium in the three-year-old trees than in the eight-year-olds (Figure 24a). However, the SFD's recorded at depths greater than 33 mm beneath the cambium in all the three-year-old trees was very low. This supports the indication, presented by the methyl orange stain, that the older sapwood was in the process of becoming dysfunctional. The figures also illustrate that there was more

variability in the SFD profiles recorded for the eight-year-old trees than in the three-year-olds. As a result, it is likely that the simulations will show that more HPV probes are required to derive accurate estimates of stand transpiration in the eight-year-old stand than the three-year-old stand.

**Table 17.** The mean and maximum sap flux densities recorded in each of the sample trees. Missing tree numbers represent trees on which HPV logger problems were experienced and therefore data was deleted.

Age class	Tree	Mean sap flux density ( $\text{cm}^3 \text{cm}^{-2} \text{hr}^{-1}$ )	Maximum sap flux density ( $\text{cm}^3 \text{cm}^{-2} \text{hr}^{-1}$ )	
3	1	59.8	124.5	
	2	75.3	173.3	
	3	69.5	190.0	
	4	77.5	241.9	
	7	46.7	131.0	
	8	59.4	195.8	
	9	62.1	115.0	
	10	46.3	134.2	
	<b>Mean <math>\pm</math> SD</b>		<b>62.09 <math>\pm</math> 10.99</b>	<b>163.21 <math>\pm</math> 41.5</b>
	8	1	52.5	161.3
2		77.9	285.4	
4		16.6	33.1	
5		68.9	259.4	
6		38.8	120.5	
7		42.0	111.2	
9		61.6	139.6	
10		33.0	72.5	
<b>Mean <math>\pm</math> SD</b>		<b>48.91 <math>\pm</math> 18.90</b>	<b>147.88 <math>\pm</math> 81.05</b>	



**Figure 24.** The mean sap flux density profile recorded in each of the three-year-old (a) and eight-year-old (b) sample trees. The curves were derived by taking the mean of the replicate SFD estimates at each depth in the sapwood for each tree.

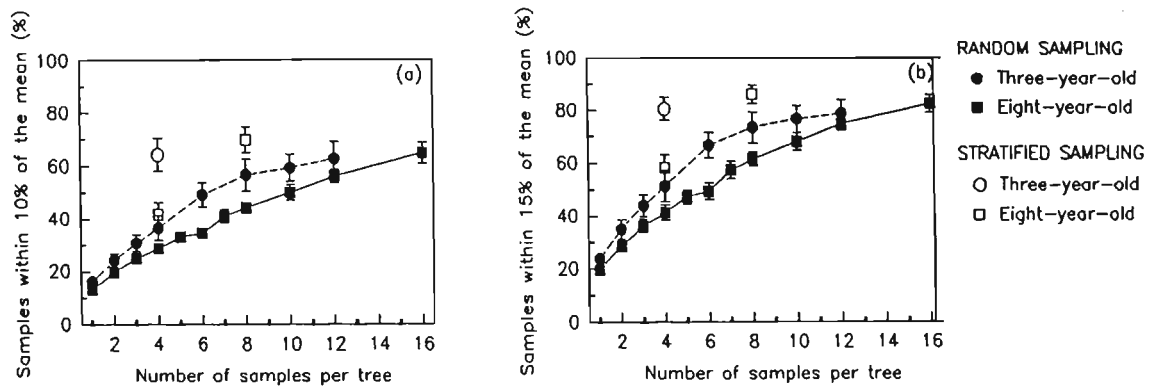
### 5.3.3 Sampling individual trees within a stand

One of the objectives of this study was to determine the sampling intensity required to gain adequately precise sapflow estimates on individual trees. A second important consideration was the issue of where the samples should be taken. Should the variation in the sapwood be sampled randomly, or should a non-random probe implantation strategy be followed?

The results of these simulations illustrate that the percentage of the samples falling within ten and fifteen percent of the true mean increases with the number of samples



selected per tree (Figure 25). The relationship is non-linear, the relative gain in terms of improved precision decreases as the number of samples per tree increases.



**Figure 25.** The relationship between the percentage of samples within ten (a) and fifteen (b) percent of the mean and the number of samples per tree. The above figures illustrate the results for both random and stratified sampling procedures in three- and eight-year-old *E. grandis* trees.

**(i) Implantation of probes to randomly selected depths**

The simulations indicated that fewer probes were required per tree to derive estimates of adequate precision in the three-year-old trees than were required for the eight-year-old trees (Figure 25). If only one probe were randomly placed in each tree, only 20% of the resulting estimates would fall within 15% of the true estimate (Figure 25). Increasing the number of probes to four resulted in an increase to 51.4% in the eight-year-old trees, and 41.3% in the three-year-trees. Increasing the sampling intensity further, to twelve per tree in the three-year-old trees and sixteen samples in the eight-year-old trees resulted in approximately eighty percent of the samples falling within 15% of the true mean.

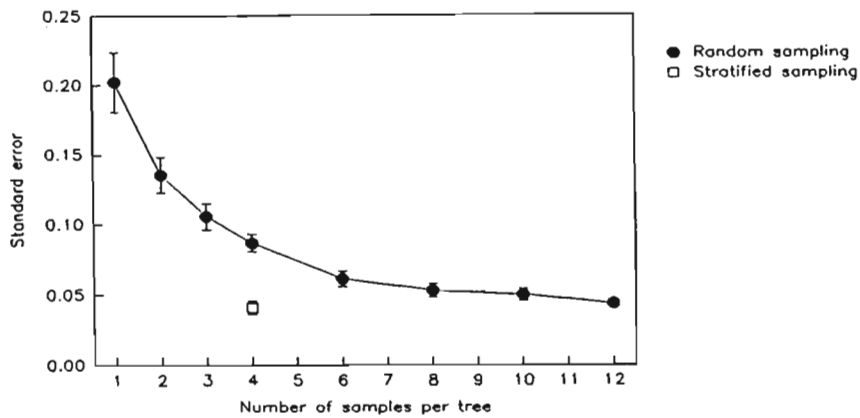
Increasing the sample intensity on individual trees also has the effect of reducing the variation observed between replicates drawn from the same tree (Figure 26 and Figure 27). The standard error of the mean sapflow estimate for each tree was used to estimate the variation between replicate estimates. In the three-year-old stand, there was relatively little variation between sample trees in the standard error of the replicate

sapflow estimates (indicated by the small standard error bars in Figure 26). By contrast, there was considerably more variation between sample trees in the eight-year-old trees (Figure 27).

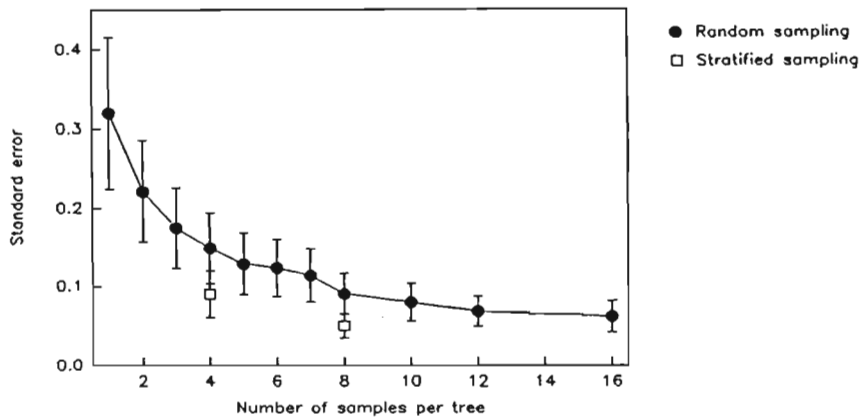
**(ii) Stratified sampling**

The practice followed in the HPV work carried out on *E. grandis* thus far was not to select the depths at random, but to stratify sampling so that each thermistor was positioned centrally within one of four annuli, each representing a quarter of the total sapwood area. The results of the simulations indicate a significant improvement in the reliability of the sample estimates derived in a stratified way by comparison to implanting probes to random depths (Figure 25). Only four probes per tree in the three-year-old trees resulted in 80.4% of the samples lying within 15% of the true mean. Eight samples per tree in the eight-year-old trees resulted in 86.0% of the samples falling within 15% of the mean. The stratified sampling approach also resulted in a significant reduction in the variation about the mean (Figure 26 and Figure 27), indicating an increased level of reliability and reproducibility.

Despite the benefits demonstrated by the stratified sampling procedure, the number of samples falling within an adequate range of the mean was still low. This is essentially for two reasons. First, both the random and the stratified sampling procedure introduced an element of bias in the estimates. The bias was introduced because the entire data set could not be used in the sampling analyses. This was particularly the case where the sapwood thickness varied around the circumference of the stem. Secondly, I feel that the fact that the sapwood of the three-year-old trees was particularly thick resulted in more variation in SFD than is normally the case.



**Figure 26.** A comparison of the mean standard error calculated for several random sampling simulations on three-year-old trees ( $N=8$ ) against the standard error of readings collected in the conventional stratified fashion.



**Figure 27.** A comparison of the mean standard error calculated for several random sampling simulations on eight-year-old trees ( $N=8$ ) against the standard error of readings collected in the conventional stratified fashion.

**(iii) *Implanting probes to a particular depth within the sapwood***

The SFD profile data presented in Figure 24 illustrates that the sap flux densities change in a semi-predictable way within the sapwood. Given an understanding of the basic structure of this variation, it is possible that a single, strategically implanted probe could give adequately precise measurements of the sap flow rate of individual trees (Swanson, 1983).

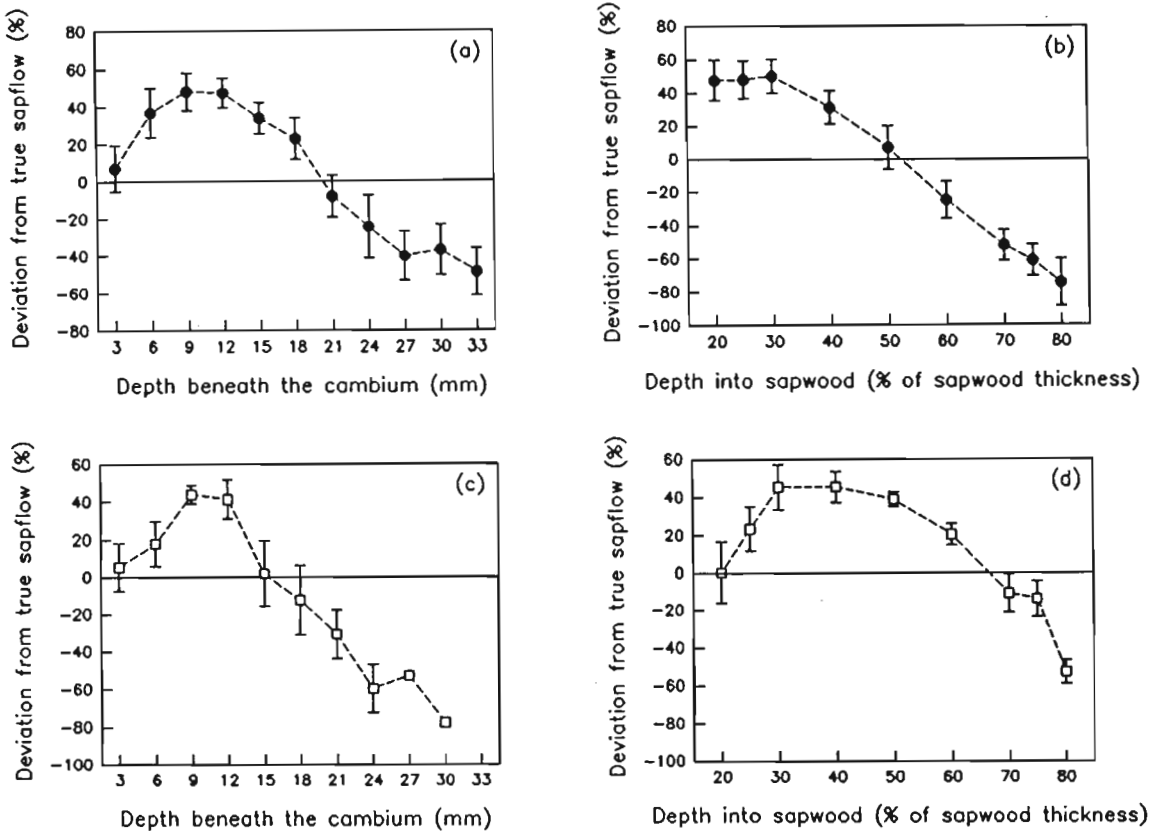
Figure 28 illustrates the consequence of implanting four probes to a particular depth within the sapwood. The mean of these four readings was used to derive a sap flow estimate and this was compared to the true sapflow of the tree. Two methods were tested: firstly, data were selected from specific depths beneath the cambium and, secondly, data were selected from depths representing specific proportions of the total sapwood available.

Both of the above strategies indicated that readings taken close to the cambium resulted in over-estimates of the sap flow, while readings selected near the heartwood resulted in under-estimates (Figure 28). The trend was similar in both the three- and the eight-year-old trees studied. Peak SFD's were recorded between nine and twelve millimetres beneath the cambium in both age classes. However, the point where average readings were recorded was not consistent between tree ages (Figure 28, b and d), and occurred over too narrow a range to be useful. This limits the potential practice of implanting probes at a particular position in the sapwood where average sap flux density readings are reliably recorded.

Two further factors militate against the approach tested above. The first is that the shape of the curve is likely to change as a function of the total sap flow rate. This may affect the depth at which average SFD's are recorded. If this is the case, the net result may be a systematic over- and under-estimation, depending on the time of day, because of the diurnal pattern in sap flow rates. The second limitation in following this sampling strategy is that it would not be clear whether the particular tree being sampled follows the general pattern illustrated in Figure 28. Tree number three in the three-year-old age class is a good example of this point (Figure 24). This tree shows a pattern which is completely different to the other seven trees sampled, and so could not be sampled with any reliability using the above techniques.

The knowledge that there is a generalised shape to the SFD profile may be of most use in sampling stands of trees rather than individual trees. In the next section, the consequences of implanting probes in several trees within a range centred on the position in the sapwood where average SFD's were recorded will be tested. This is

proposed as a potential refinement on the option where probes are simply implanted at random over the entire sapwood.



**Figure 28.** The mean deviation from the true sapflow estimated at various depths in the sapwood, observed for eight trees per age-group. Graphs (a) and (b) present the data for three-year-old trees, while (c) and (d) present the data for eight-year-old *E. grandis* trees. Measurements were made at increasing depths below the cambium. Results have been expressed in two ways: firstly the absolute depth beneath the cambium (a) and (c) and, secondly, the depth at which readings were taken was expressed as a proportion of the total sapwood thickness (b) and (d).

### 5.3.4 Estimating transpiration from stands of trees

#### (i) Three-year-old stand of *E. grandis*

The simulations carried out on the three-year-old trees indicate that weighted estimates resulted in marginally more samples lying within 15% of the true stand value (Figure 29b), and had a lower standard error estimate (Figure 29d), indicating a higher degree of reproducibility. The most reliable probe depth allocation strategy was again

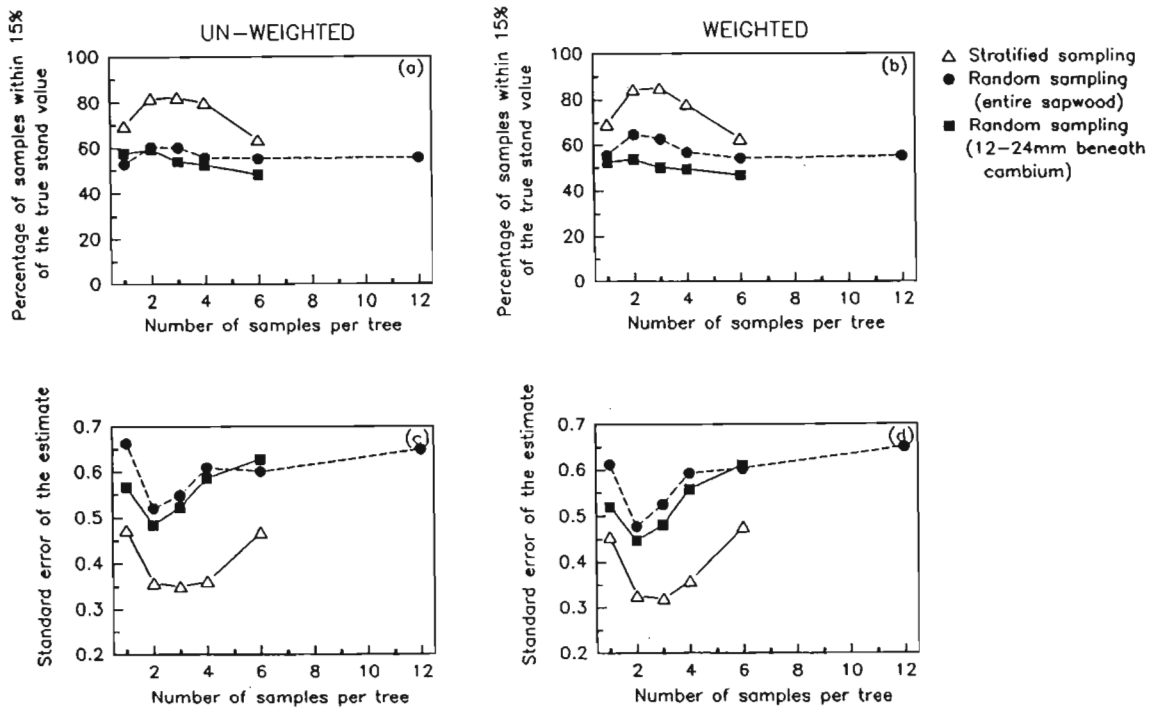
found to be a stratified approach. The next most reliable was the random strategy, followed by random allocation to a restricted depth within the sapwood centred about the point where mean SFD's were generally recorded (Figure 29).

All three strategies tested showed the same basic trend: increasing the number of probes per tree and decreasing the number of trees sampled resulted in progressively fewer samples lying within 15% of the true stand value, and a progressively higher standard error (Figure 29b and d). The most dramatic changes were experienced over the range from two to six samples per tree, whereafter the curve flattened out (Figure 29). This indicates that there was a significant advantage to sampling more than four trees per stand, particularly if the weighted approach was used.

The apparent dramatic difference between the 'one sample per tree' and 'two samples per tree' simulation is at least partially due to the fact that the sampling intensity for the stand was not equal in both cases (Figure 29). Because only eight trees were available for the simulation, the 'one sample per tree' case has only a total of eight samples. The 'two samples per tree' has a total of twelve samples, as it was possible to select two readings from each of six trees. The way in which the analysis was done therefore precludes an interpretation of difference between these two cases.

The most reliable stand estimates were recorded in weighted simulations where three probes were implanted in each of four trees and two in each of six trees (Figure 29b and d), following the stratified approach with depth. If data were selected using either of the random approaches, then the most reliable results were attained with two probes per tree, although these were significantly poorer than the results from the stratified approach.

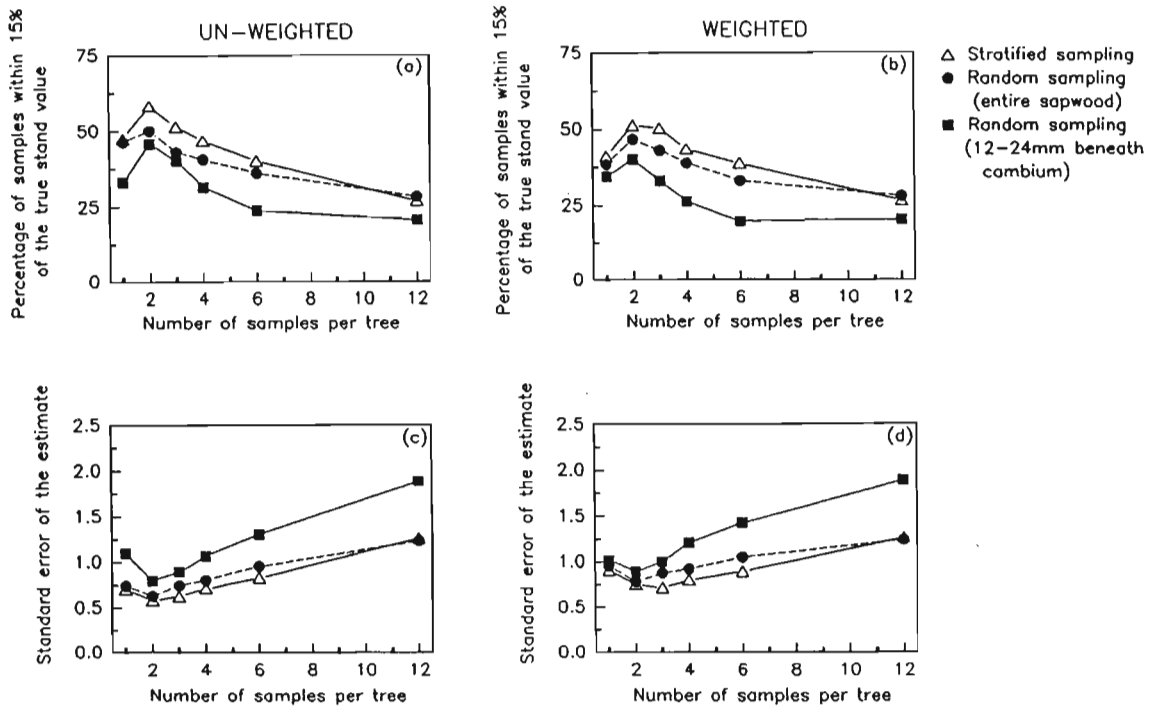
The stratified sampling routines' simulated data were normally distributed about a value 5.58% lower than the true stand mean. Because of this bias, the results for the percentage of samples falling within 15% of the true stand transpiration have been presented in Figure 29. The limits are a little lenient, so as to allow some compensation for the fact that simulation introduced a slight bias.



**Figure 29.** The relationship between the number of samples per tree and the number of samples within 15% of the true transpiration rate for a stand of three-year-old *E. grandis*, for unweighted (a) and weighted simulations (b). The figure also illustrates the relationship between the standard error of the mean predicted value and the number of samples per tree. The total number of samples per stand was held constant at 12, except for the simulation of one sample per tree, where the total number of samples was eight.

**(ii) Eight-year-old stand**

Results for the eight-year-old trees follow the same basic trends as those for the three-year-old trees sampled. Again, the best sampling routine was the one in which the probes were implanted to stratified depths, followed by the two random sampling routines tested (Figure 30). The relative difference between the various strategies of implanting the probes with depth was not as marked in the eight-year-old stand as in the three-year-old stand (Figure 29, Figure 30). However, implanting the probes to stratified depths remained consistently the best probe implantation strategy.



**Figure 30.** The relationship between the number of samples per tree and the number of samples within 15% of the true transpiration rate of the eight-year-old *E. grandis* stand, for the unweighted (a) and weighted simulations (b). The figure also illustrates the relationship between the standard error of the mean predicted value and the number of samples per tree. The total number of samples per stand was held constant at 12, except for the simulation of one sample per tree, where the total number of samples was eight.

In contrast to the three-year-old stand, in the eight-year-old stand the unweighted analyses resulted in the largest number of samples falling within 15% of the true stand value (Figure 30). The most reliable stand transpiration estimates were derived from the simulation with two samples per tree in each of six trees (Figure 30a) with the probes implanted to stratified depths. This resulted in 58.2% of the samples lying within 15% of the true stand value.



### 5.3.5 Assessing the accuracy of the sampling strategy used in the age-sequence study.

The results of the simulations of the accuracy of the sampling strategy used in the age sequence study are presented in Table 18. They illustrate that there was a higher precision associated with the predicted stand transpiration rates than there was for the individual trees within a stand. This level of precision could be expected to decline as tree age (and size) increased, as was the case in the age sequence study, because a maximum of eight probes were used per tree. Because there was considerably more precision associated with the stand estimates, only the mean stand response was modelled in the age sequence chapter.

**Table 18.** A summary of the accuracy with which transpiration was estimated using the sampling strategy employed in the age-sequence study. The table presents data for the accuracy with which both transpiration from individual trees and stand transpiration were predicted. The bias introduced in the simulated estimates which are associated with the sampling optimisation data, rather than the age-sequence data, is also presented.

Age-class	Number of probes per tree	Number of trees sampled	Individual tree transpiration			Stand transpiration		
			Percent samples within 10% of the true mean	Percent samples within 15% of the true mean	Bias in the simulated estimate	Percent samples within 10% of the true stand value	Percent samples within 15% of true stand value	Bias in the simulated estimate
3	4	5	64.1	80.4	1.7%	79.7	95.0	-1.3%
8	4	5	41.8	58.2	2.7%	47.6	66.2	4.7%
8	8	5	69.6	86.0	5.8%	79.3	96.1	2.7%

## 5.4 DISCUSSION

The results of these simulations clearly illustrate the benefits of using a stratified probe implantation strategy in *E. grandis*. This strategy was found to give significantly more accurate and repeatable results than if the probes were either implanted to random depths in the sapwood, or to a particular position in the sapwood. Four probes implanted to stratified depths in three-year-old trees resulted in a mean of 64.1% of the

samples lying within 10% of the true mean. Eight probes in the eight-year-old trees resulted in 69.6% of the samples lying within 10% of the true mean. By comparison to these results for *E. grandis*, the same sampling intensities on *P. patula* were found to return poorer estimates of reliability (Dye *et al*, 1991). Four probes resulted in a mean of 51.2%, and eight probes resulted in 65.3% of the samples lying within 10% of the true mean value.

The increased sampling requirement in *P. patula* is apparently due to the rather thick sapwood (often in excess of 80 mm) and seasonal growth rings which result in an undulating SFD profile. For this reason, Dye *et al.* (1991) proposed that probes be implanted in a stratified random way. The sapwood area is divided into two sectors, the outermost 75% and the innermost 25%. The replicate sensors should then be implanted to random depths within each of the sectors, proportionally more sensors being implanted in the outer sector than the inner sector. In this way the sampling is stratified, but the depth of each probe within each sector is selected at random.

The best probe implantation strategy for **individual trees** of *E. grandis* resulted in 80.4% and 86.0% of the samples falling within 15% of the true mean in the three- and eight-year-old trees respectively. These results were achieved using four probes in the three-year-old trees and eight probes in the eight-year-olds, which is well within the physical limitations described above. The simulations also illustrated that increasing the number of probes achieved better results.

There is a physical limitation to the number of probes that can be implanted around the stem of a tree. Swanson (1983) recommended that probes be separated by at least 40 mm laterally and 100 mm vertically, to avoid interference between different pulses of heat. However, the limitation arises more from the occurrence of knots and branchlets on the stem of young *E. grandis* trees, than Swanson's criterion. Knots and branchlets may influence the sap flow path in the sapwood in their immediate vicinity, and therefore I always avoid implanting probes close to knots or branchlets. In practice, it is not really feasible to implant more than six probes on a 12 cm diameter

stem (typical of three-year-old trees). The occurrence of deformities in the stem also affects the number of probes one can implant.

Another option would be to implant probes at more than one level on the stem of the tree. However, I am not in favour of implanting probes on more than one plane on the tree because experience has shown that the wound response extends at least 100 mm vertically from the probe implantation site on *E. grandis*. It is not clear to what extent the upstream wounds interfere with measurements of downstream sapflow. For this reason it is instructive to examine what precision is achieved given the limitation to the number of potential probe implantation sites around the stem of a tree. Also, the slight bias introduced in the analyses distorts the overall view. For this reason, the results have also presented the proportion of samples within 15% of the mean. I believe that this resolution is still biologically meaningful, especially in studies such as this one where results are often extrapolated over large areas where potentially greater inaccuracies are introduced by the other unknowns in the scaling procedure.

The simulations of **stand transpiration** present an important lesson: the more trees that are sampled the better. This was the approach used by Swanson<sup>3</sup> (pers. comm.) in sampling spruce stands in Canada, although he had not tested it. His approach was to implant one probe in as many trees as possible, and use the mean of these estimates to calculate the stand transpiration. In the simulations conducted on *E. grandis* the best results were achieved by implanting two probes in each of six trees in the eight-year-old stand, and either two or three probes per tree in the three-year-old stand. Again, a stratified approach produced consistently more accurate and repeatable results.

The best simulation resulted in 84.9% and 58.2% of the samples falling within 15% of the true stand transpiration in the three- and eight-year-old stands respectively. It is important to emphasise that the question being addressed was: how should 12 probes be used in the estimation of stand transpiration? However, the sapwood area sampled

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<sup>3</sup> Swanson, R.H. Canadian Forestry Service, 5320 - 122nd Street, Edmonton, Alberta, Canada

in the eight-year-old stand was about 50% larger than the three-year-old stand, and the two results are therefore not directly comparable. Given that, at best, only 58.2% of the samples fall within 15% of the true value, more than 12 probes are required to sample eight-year-old stands accurately. The relationship between number of samples and accuracy is non-linear (Figure 25), implying that at least 18 probes are needed to raise the percentage of samples within 15% of the mean to over 80%.

This simulation exercise was approached with an aim to realistically simulate the equipment we currently use. However, simulations where more than one reading was selected per radius effectively simulates cases where multiple thermistors are implanted in each probe. One way of overcoming the physical limitation regarding how many probe implantation holes can be drilled in the stem would be to increase the number of samples per probe. Another way, practised by Israeli heat pulse workers (eg. Cohen *et al.*, 1981), is to implant a heat-conducting strip in the probe, against the temperature sensor. Swanson uses a similar system where the temperature sensors are embedded in a brass tube 10 mm long. The net result in both these cases would be to increase the sphere of sensitivity of the temperature sensors, thereby imparting some kind of averaging effect over a section of the sapwood equal to the length of the heat conducting strip. This is desirable in that it smooths out some of the variation illustrated between successive readings in Figure 24. What is not clear, however, is whether this approach results in an unbiased average. I would expect that the sensor is likely to respond to sections where the heat arrives first, introducing a positive bias.

The field data collection was carried out over the midday period when maximum flow rates are generally recorded. During this time of day, the difference between the SFD's recorded by deep and shallow probes is most accentuated. As a result, within-radius variation in SFD is at its greatest, resulting in conservative estimates of the sampling requirements. However, despite the fact that the simulations are likely to produce conservative estimates, because the greatest flux rates are recorded at mid-day, this is the time of day for which the most accurate transpiration estimates are

required. A relatively small percentage error at this time of day could potentially result in a serious error in the estimation of total daily transpiration.

During times of low sap flow, I would anticipate that within-radius variation would be reduced, with the consequence that fewer probes be required to make accurate estimates of sap flow. I also feel that the unusually thick sapwood in the three-year-old trees resulted in more within-radius variation than is typically the case, again resulting in conservative estimates of the accuracy of the technique at any given sampling intensity.

It is not clear whether the difference in required sampling intensity observed between the three- and eight-year-old trees and stands was a consequence of the fact that the trees were older, or a consequence of the fact that relatively more sapwood was sampled in the eight-year-old stand. One of the sources of variation within the stands is undoubtedly genetic. Significant differences in transpiration have been illustrated between genotypes and it would be interesting to establish whether clones have less variation in SFD's between trees than do open-pollinated seedlings.

Valuable insights into the probe requirements for sampling transpiration from individual trees and stands of trees were gained from this study. Two important concepts have emerged: firstly, there is a clear benefit to stratifying the depths to which probes are implanted in *E. grandis*, this technique results in greater precision and repeatability in the HPV-derived estimates of sap flow in *E. grandis*. Secondly, given a limitation in the number of probes available to estimate stand transpiration, it is desirable to measure many individuals with a low sampling intensity rather than follow the same approach used to measure individual trees.

## CHAPTER 6.

### GENERAL DISCUSSION

This thesis has dealt with several issues relating to the heat pulse velocity technique and specifically its application to the study of transpiration from an age sequence of *E. grandis*. The work done exploits the technological advance from leaf-based techniques, such as porometry, to whole-tree based measurements permitted by the use of an instrument that measures the flux of water through the stem of plants.

The physical and anatomical features of *E. grandis* make it an ideal species for the application of the heat pulse velocity technique. The relatively thin bark and generally narrow sapwood band implies that long probes are not required. This simplifies both the drilling and probe implantation process. In addition the stems are generally not fluted, making the calculation of sapwood cross-sectional area a simple matter. Anatomically, *E. grandis* is a diffuse porous hardwood and growth rings are either only vaguely evident or absent altogether (Malan, 1988). This means that the sampling problems associated with growth rings are avoided (Dye *et al.*, 1991). The wound response is clearly visible, facilitating the accurate measurement of this important variable.

*E. grandis* showed a tight coupling between sapflow measured at the base of the stem and meteorologically-induced transpiration changes. This indicates very low resistance to sap flow in the stem of the tree, a fact corroborated by the observation that if trees are felled and their canopies removed, the water flows freely from the downhill cut surface. However, additional research is required to verify that there is minimal

capacitance in *E. grandis*. This would require several 24-hour runs under different weather conditions, although the low flow rates likely during night-time recharge (if there is stem capacitance) are likely to be beyond the resolution of the HPV technique.

The apparent lack of lags between changes in the sap flow rate measured in the stem and transpiration from the canopy, enabled the relationship between instantaneous transpiration rates and synchronised meteorological data to be modelled. If a capacitance effect was evident, a different modelling approach would have been needed. In such a case, total daily sap flow, rather than instantaneous transpiration, would need to be modelled against daily PAR and VPD.

The verification of the apparatus indicated that it was relatively easy to gain estimates that appeared to be realistic, but were actually quite incorrect. Even if the wound size were not measured, and an average assumed, the data analyses would produce results that appeared qualitatively correct. Estimated sap flow rates would be low at night and rise sharply as PAR levels increased in the morning. On clear days the transpiration rates would peak a little after solar noon and this would be followed by a general decline towards evening. Because the first application of the technique to trees produces data that appear realistic, it is tempting to forsake testing the apparatus on the species for which it is to be used, and proceed directly with the application of the technique. Herein lies the greatest threat to the credibility of the technique - it is easier to apply than apply properly.

Measuring the speed at which the heat pulse moves has become technically easy, hence the automation of the instrument. However, converting the heat pulse velocity to an accurate estimate of sap flux requires some thought. The sensitivity analyses conducted in this thesis illustrates the critical importance of accurate wound size estimates. Inaccurate estimates can lead to serious errors in the estimation of sap flux. For this reason, I suggest that the wound sizes be directly measured following the use of the instrument. Again, general trends in wound size are evident and it is tempting to estimate rather than measure this parameter. My experience has been that variation in wound size does occur, particularly through the mal-alignment of probes in the

vertical plane and in trees which have a spiral grain. This problem can be serious especially if probes are implanted to some depth within the sapwood.

The positioning of probes in the sapwood is also an area in which particular care should be taken. Probe implantation should aim to achieve an unbiased estimate of sap flow and should also not violate the assumptions emphasised by Swanson (1983) regarding their separation from one another. For this reason I investigated alternate probe implantation strategies, as described in Chapter 5. The results indicated that the most desirable probe implantation strategy was to stratify sampling through the sapwood of *E. grandis*, giving each probe equal weight. This is not always the optimal solution. In trees with growth rings, such as *Pinus patula*, we found that the implantation of probes to random depths was a more robust sampling procedure (Dye *et al.*, 1991). The issue here is that the sampling strategy needs to be matched with anatomy of the tree being sampled, and ultimately, also an understanding of where the major sap flux is occurring. Neither of the above approaches may be appropriate for *Quercus spp.* which develop a very pronounced ring structure.

An area that is often treated only cursorily is the matter of how the individual estimates of sap flux density are integrated to derive an estimate of total sap flow. One approach often used is to fit a quadratic function to the sap flux density estimates made at depths through the sapwood, integrate the area under this fitted curve and multiply by the sapwood area to derive an estimate of transpiration. I contend that this procedure is not adequately verified and that the quadratic function does not realistically fit the sap flux density profile. This is definitely the case for ring-porous trees such as *P. patula* (Dye *et al.*, 1991) and also the case for the *E. grandis* trees sampled in this study. In *E. grandis* the profile is skewed, maximum rates generally occurring close to the outer section of the sapwood. In most cases a quadratic function would not fit the profile well. I suggest that the average of the individual samples be used in calculating the transpiration rate. The examples cited demonstrate the value of establishing the nature of the radial sap flux density profile.



The importance of sampling stands of trees became clear during the analysis of the age sequence data. It is inevitable that experimental procedures can be improved in hind sight. However, at the time the age sequence study was conducted, only two HPV loggers were available each of which could handle four heat pulse probes. These were used to maximum benefit in the rotational sampling procedure used. However, the primary aim of the study was whether the transpiration of stands of trees changes with age and season. Although the matter was successfully addressed, despite the limitations of the rotational sampling procedure, it would have been statistically easier to draw conclusions had all the age classes been studied on the same day. The sampling optimisation study showed that this would be possible with relatively limited apparatus, provided the loggers could handle more than just four probes. The loggers we now use have a capacity to handle up to 12 probes each. Two of these loggers could therefore be deployed in a stand with a single thermistor in each of twenty-four trees to gain a much better estimate of stand transpiration than was obtained by sampling each of the sample trees as heavily as was the case in the age-sequence study. These sampling techniques need to be considered in future applications of the technique to stands of trees.

Despite the limitations of the apparatus on hand to do the age-sequence study, the results were significant in terms of the prediction of the hydrological impact of plantation forestry. It could not have been anticipated that there would be such a dramatic difference in the transpiration rates of young and old *E. grandis* stands. This result was surprising as the LAI of the stands does not vary that significantly over the range of ages tested, and even the oldest trees did not display symptoms of water stress.

The models developed in this thesis have aimed at estimating transpiration per unit leaf area from *E. grandis* trees. This is consistent with other recognised process models such as that developed by Running and co-workers (Running and Coughlan, 1988). To predict transpiration in mm, the model output would be multiplied by an estimate of the LAI. However, not only water stress affects the LAI of trees (Waring and Schlessinger, 1985), and it is possible that the coefficient particularly of the PAR term

in the model may change as LAI increases. It is important to bear this possible limitation in mind when applying the model to stands with LAI's in excess of the stands on which the model was developed.

It would be interesting to investigate to what extent *E. grandis* adjusts its leaf area to avoid water stress, as opposed to regulating its stomatal aperture under declining leaf water potential. Work by Connor *et al.* (1977) demonstrated that the stomata on eucalypts could remain open even at water potentials of 2.4 MPa. If *E. grandis* preferentially employs a strategy of reducing its leaf area to avoid water stress, it is possible that the relationship between transpiration per unit leaf area and the two key driving variables may be quite stable. If this is the case, then the model may be applicable even to stands of low LAI and possibly even moisture-stressed stands.

It was surprising that a simple linear multiple regression accounted for as much of the observed variation in the age sequence data, given the demonstrated non-linearity of the relationship between stomatal conductance and the driving variables (Dye and Olbrich, 1993; Myers and Neales, 1984). Although there is some doubt as to the validity of some of the transpiration estimates derived using the linear models, there was no evidence in the data collected that the limit of the linearity had been reached. This is an interesting feature of *E. grandis* and may well reflect the efficient conducting system it has, and the conditions pertaining at the time of data collection. Had there been a measure of water stress the relationship may well have started demonstrating the feed-forward inhibition of transpiration cited in the literature (Cowan, 1977). Work is currently under way to investigate transpiration from *E. grandis* under water stress. This should provide the answer to the above question.

The simulation exercise showed that transpiration rates in winter were between 25 and 50% lower than in summer. This was attributed to the combined effect of the seasonal shift in the response function, the shorter day length in winter and the lower levels of insolation. The mechanism responsible for the seasonal change in the response function requires further investigation. The result is, however, fortuitous in that it suggests that the hydrological impact of *E. grandis* plantations is least marked during

the dry winter months when competition for water resources is most severe. Less desirable is the sharp rise in transpiration recorded in September, at a time coinciding with the climax in competition for water because this precedes the summer rains.

The simulation exercises conducted illuminated the dramatic nature of the differences between stands of increasing age, sixteen-year-old stands were estimated to transpire only 40% of the amount estimated for three-year-old trees under the same climatic conditions. The most plausible explanation for these observed differences is that as the trees grow older their water conducting system cannot meet the demand. However, if this is the mechanism, then one would expect the older trees to show some symptoms of water stress. These may include elevated pre-dawn XPP levels or an indication of mid-day stomatal closure on clear days with relatively high evaporative demand. Neither of these symptoms was however present. There may therefore be a physiological mechanism that modulates the response function to avoid the stress situation arising in the first place. The mechanism behind the decline in transpiration with age requires further study to substantiate the theories for its occurrence.

These results imply that the short rotations commonly practised in the forestry sectors growing timber for pulp are likely to have a more dramatic hydrological impact than those growing timber under the longer saw-log rotations. However, it is necessary to look at the matter more holistically, because the importance of under-story transpiration is likely to escalate as the stands grow older, offsetting a decline in transpiration from the plantation trees. Also, the predictions of high daily transpiration need to be validated on an independent data set before the absolute differences can be modelled.

The data presented in the thesis have illustrated a strong age-related effect. The effects demonstrated have been of quite a dramatic nature given the range of ages studied relative to the longevity of trees. The changes attributed to increasing tree age may well be a consequence of tree size rather than age, and it is possible that the time-frame of the observed changes is compressed merely because of the rapid growth rates experienced in the Sabie region. Consequently, the observed changes

may take longer to manifest themselves in slower growing *E. grandis* trees, particularly if the change is a consequence of deteriorating vascular functioning as a result of the long flow paths in large trees as suggested by Cole *et al.* (1990).

In conclusion this thesis has shown that the heat pulse velocity apparatus is a suitable instrument for measuring water use in *E. grandis*. The limitations of the technique have been outlined and recommendations made to refine its application to *E. grandis*. The water use of *E. grandis* has been shown to vary with both age and season, with net rates declining with tree age, and winter rates of water use being lower than summer rates under similar conditions. Through the use of this technique substantial advances have been made towards predicting water use from plantation eucalypts, and therefore improving the hydrological management of afforested catchments.

## CHAPTER 7.

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## The verification of the heat pulse velocity technique for estimating sap flow in *Eucalyptus grandis*

BERNARD W. OLBRICH

Division of Forest Science and Technology, Council for Scientific and Industrial Research, Private Bag X520, Sabie 1260, South Africa

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The heat pulse velocity technique was verified on a diffuse porous hardwood, *Eucalyptus grandis* Hill ex. Maiden, using the cut-tree method. The heat pulse velocity apparatus accurately reflected sap flow in the stems of 3-year-old trees and of a 16-year-old tree. Measured sap flow was found to be particularly sensitive to errors in the estimation of the wound size and probe separation. Four probes, each providing a point estimate of heat pulse velocity, were sufficient to estimate sap flow in young trees, but a minimum of eight was needed for trees larger than about 20 cm in diameter. For the successful field application of the heat pulse velocity technique on standing *E. grandis* trees, it is necessary to wait 5 days after implanting the probes to enable tyloses to form, which facilitates wound size measurement. This technique is recommended for the accurate and rapid measurement of water use by *E. grandis* and other similar plantation tree species.

OLBRICH, B. W. 1991. The verification of the heat pulse velocity technique for estimating sap flow in *Eucalyptus grandis*. *Can. J. For. Res.* 21 : 836-841.

La technique de vitesse de pulses de chaleur a été testée sur un feuillu à pores diffus, *Eucalyptus grandis* Hill ex. Maiden, par la méthode de l'arbre coupé. Les mesures obtenues avec l'appareil de vitesse de pulses de chaleur reflétaient avec exactitude l'écoulement de la sève dans le tronc d'arbres de 3 ans, et d'un arbre de 16 ans. L'écoulement de la sève mesuré était particulièrement sensible aux erreurs dans les estimations de la taille de la blessure et de l'intervalle de séparation des sondes. Quatre sondes, chacune donnant un estimé ponctuel de la vitesse du pulse de chaleur, suffisaient à mesurer l'écoulement de la sève dans les jeunes tiges. Un minimum de huit sondes étaient requises pour les tiges avec un diamètre supérieur à 20 cm. Afin d'assurer le succès de la technique de vitesse de pulses de chaleur sur des tiges non coupées de *E. grandis* sur le terrain, on doit donc attendre 5 jours pour permettre aux tyloses de se former, ce qui facilite la mesure la taille des blessures. Cette technique est recommandée pour obtenir une mesure rapide et exacte de l'utilisation en eau par *E. grandis* ou par d'autres arbres de plantation du même type.

[Traduit par la rédaction]

### Introduction

The measurement of forest transpiration remains a difficult task despite recent advances in instrumentation and progress with micrometeorological approaches. Most techniques require a structure either to gain access to the canopy or to serve as a platform for micrometeorological instrumentation mounted above the canopy. Leaf-based techniques such as porometry face additional problems in sampling the canopy adequately to allow the estimation of whole-tree transpiration rates. Bowen ratio and eddy correlation have theoretical constraints that are difficult to satisfy in undulating, discontinuous forest terrain. There is thus an obvious need for "a rapid, simple method for measuring tree transpiration in forest hydrology..." (Swanson and Whitfield 1981). Since all water transpired from the canopy must pass through the stem, this is the logical site for measurement. Estimates of the quantity of water passing up the stem can be made using the heat pulse velocity (HPV) technique (Swanson 1983). This method is rapid and amenable to automatic data capture, and the instrumentation is portable, allowing estimates on a number of trees during the course of a day.

The concept of using a pulse of heat as a tracer in the sap stream of trees to measure transpiration is not new (Huber and Schmidt 1937). Until recently, however, it was not possible to apply the HPV technique to trees without prior calibration (Cohen *et al.* 1981; Marshall 1958; Swanson 1983). The implantation of probes in the stem and

the resultant interruption of flow result in a violation of the assumptions of idealized heat transport (Swanson and Whitfield 1981), and it becomes necessary to correct for this by measuring the width of the zone where flow has been interrupted (wound size). Accurate transpiration estimates have been attained by workers for *Nothofagus solandri* (Hook.f.) Orst., *Pinus halepensis* Mill., and *Pinus radiata* D. Don using the HPV technique with a correction factor for the wound size measured on completion of the experiment (Swanson and Whitfield 1981).

The present paper outlines the verification and application of the HPV technique to a diffuse porous hardwood species, *Eucalyptus grandis* Hill ex Maiden, the major plantation-grown hardwood in South Africa.

The specific objectives of this study were (i) to investigate how accurately the HPV technique could measure the amount of water flowing up the stems of two age-classes of *E. grandis* trees and (ii) to determine the sensitivity to errors in the measurement of the various parameters required to calculate sap flow.

### Site description

Trees were selected from stands of plantation-grown *E. grandis* on Frankfort State Forest in the Eastern Transvaal, South Africa (25°03'S and 30°53'E). In this area *E. grandis* trees are fast growing, and growth rates in excess of 5 m per year are not uncommon. The area is situated

1100 m above sea level, has a mean annual temperature of 18.1°C, and receives an average of 1230 mm rainfall per annum falling predominantly in the summer months. The soils in the area are typically granite-derived, deep, red Ferrasols of the Hutton form (McVicar *et al.* 1977).

### Materials and methods

#### HPV data analysis

The verification experiments were carried out using a custom heat pulse velocity recorder (Soil Conservation Centre, P.O. Box 8041, Palmerston North, New Zealand). The system includes a data logger with four sets of probes, each of which gives a point estimate of HPV.

The four pairs of Teflon probes with a single thermistor in each probe are connected in a Wheatstone bridge configuration and adjusted to zero output before generating the heat pulse. The heat pulse is generated by supplying a current to the four line heaters for 0.7 s, and the time taken for the bridge to return to the initial balance point after the heater is pulsed is then recorded.

In this verification study the following procedure was followed. The HPV ( $u$ ) was calculated using the following formula (Swanson 1983):

$$[1] \quad u = (X_u + X_d)/2t$$

where  $X_u$  and  $X_d$  are distances from the line heater to the upstream and downstream thermistors, respectively;  $(X_u + X_d)/2$  is referred to as the probe separation in the remainder of this paper, and  $t$  is the time taken for the bridge to return to the initial balance point after the heater is pulsed.

The corrected HPV ( $u'$ ) are then calculated using Swanson and Whitfield's (1981) wound correction coefficients. The correction takes the form

$$[2] \quad u' = p + qu + r(u)^2$$

where  $p$ ,  $q$ , and  $r$  are the correction coefficients derived for the measured wound size and Teflon probes in the (-0.5, 0, 1.0) configuration.

The corrected HPV are converted to sap flux (Marshall 1958):

$$[3] \quad v = \rho_b(m_c + 0.33)u'$$

where

- $v$  is the sap flux
- $\rho_b$  is the wood density
- $m_c$  is the moisture fraction, dry weight basis
- 0.33 is the specific heat of dry wood

Therefore

$$[4] \quad \text{sap flow} = vA$$

where  $A$  = sapwood area. The moisture fraction in eq. 3 can be calculated using

$$[5] \quad m_c = (\text{fresh weight} - \text{dry weight})/\text{dry weight}$$

In this study on *E. grandis*, the moisture fraction was measured in four sapwood samples from each tree, which were first weighed fresh and subsequently dried in an oven at approximately 80°C until no further weight loss was recorded.

The wood density,  $\rho_b$ , in eq. 3 was calculated using

$$[6] \quad \rho_b = \text{dry weight}/\text{volume of fresh wood}$$

The volume of the fresh wood sample was determined by

immersion in a beaker of water and the application of Archimedes' principle.

Since radial differences in sap flux do occur (Swanson 1983), each of the four probes was used to derive an estimate of sap flux considered to represent that at a position in the sapwood. The sapwood area associated with each probe was then calculated on the basis of four concentric rings with their limits midway between successive thermistors. Total sap flow was calculated as the sum of these partial areas multiplied by their associated sap fluxes. This method follows that of Swanson (1983) and is referred to as the step function in this paper.

#### Cut-tree procedure

The HPV estimates of water use of 3- to 4- and 16-year-old trees were compared with a direct measure of water uptake recorded during a series of cut-tree experiments. This technique (Roberts 1977) entails cutting a tree at its base and inserting it in a bucket of water. Verification involved measuring direct water uptake from the bucket and comparing this with uptake calculated from simultaneous HPV measurements.

#### Three-year-old trees

The trees selected were typically 17 to 20 m tall, carried a leaf area of 50 to 80 m<sup>2</sup>, and had a diameter at cambium of about 13 cm (at 1.3 m). Prior to cutting, the tree was tied to a supporting tripod and tethered to neighbouring trees for stability (Roberts 1977; Knight *et al.* 1981). Immediately after cutting, the cut end was inserted in a bucket with water. The entire cutting procedure took approximately 30 s. Cutting was performed before dawn, when water potential was highest, and the exposure of the cut end was kept to a minimum to prevent entry of air into the vessels.

An aqueous coloured dye was added to the water in the bucket to examine the pattern of water uptake in the sapwood. A trial run indicated the necessity of making a fresh cut under water with a sharp, broad-bladed chisel to prevent the occurrence of vessel blockages. This was shown to be successful since it resulted in an even distribution of dye throughout the sapwood. All bark was also removed from the section of the stem in the bucket to prevent the introduction of phloem exudates into the water (Green and Clothier 1988).

#### Sixteen-year-old tree

The tree selected was 56 m tall, had a diameter of 41.2 and 35.0 cm at cambium and heartwood, respectively (at 1.3 m), and had a leaf area of 219.3 m<sup>2</sup>. The cut-tree method was modified for the larger tree, as a tripod support was not practical. Prior to cutting, the bark was removed from the basal section of the stem. A flexible, water-tight container was made by wrapping a plastic sheet around the stem and sealing it at the bottom and side where the sheet overlapped. The tree was left standing, and a girdling cut (approximately 5 cm deep) was made using a chain saw through the sapwood to the heartwood around the entire circumference of the tree. The heartwood was thus left intact to support the tree. A second cut was made about 5 cm beneath the first, and the section between the two cuts was removed to facilitate access to the lower sapwood surface. This surface was then sealed with molten candle wax to prevent basipetal movement of water. The container was pulled downwards during cutting and raised and filled with water immediately

TABLE 1. The dimensions and leaf areas of the trees used in the verification of the HPV technique on *E. grandis* and Apan evaporation rates during the experiments

Tree No.	Age (years)	Height (m)	Diameter (cm)		Sapwood area (cm <sup>2</sup> )	Leaf area (m <sup>2</sup> )	Apan (mm·d <sup>-1</sup> )
			Cambium	Heartwood			
1	3	—	12.1	5.4	92.9	—	4.0
2	3	19.5	13.6	8.8	84.7	53.69	3.5
3	3	17.9	12.3	8.4	62.7	40.17	3.5
4	3	18.5	15.5	10.8	97.1	71.43	5.0
5	16	56.0	41.2	35.0	371.1	219.30	5.0

after the cutting had been completed. A metal strip was riveted to the upper rim of the container, forming a rigid frame from which the container was suspended in the raised position. The upper sapwood surface was then recut underwater with a sharp chisel to remove any blockages. Water uptake from the container was measured by refilling it to a fixed mark every 10 or 15 mins.

#### Wound size measurement

On completion of each experiment the sections of the tree trunk containing the probe implantation holes were excised from the stem. Each sample was shaved with a microtome, and the wound width was measured with an ocular graticule on a dissecting microscope. The wound was identified as that section of the sapwood in which tyloses had been deposited in the vessels. Measurements were taken midway between the line heater element and both the upper and lower thermistors. Eight readings were taken on each sample, making a total of 32 estimates for each set of four probes. A single average was then used as the final wound width estimate for each tree.

#### Probe placement and implantation

Preliminary investigation showed that the sapwood in both age-classes of *E. grandis* is about 30 mm thick. The placement of probes was biased towards the outside since this is where sap flux is highest and it accounts for a relatively greater sapwood area. The probe depths within the sapwood were thus set at 4, 10, 17, and 23 mm beneath the cambium for both size classes.

Each probe was implanted in a different quadrant around the circumference of the tree to account for variation around the stem. Deformities in the stem were avoided. In the larger tree, it was possible to divide the outer surface into eight sectors and each of these into four parts. Each probe was then allocated randomly first to a sector and then to a part within that sector.

The probes were inserted through holes drilled radially in the stem at a height of approximately 1.3 m. The access holes were drilled with a battery-operated hand drill fitted with a 1.85-mm drill bit. The 1.75 mm thick probes were implanted in the (-0.5, 0, 1.0) configuration (Swanson 1983). A 20 mm thick drill jig was used to ensure that the holes were correctly spaced and parallel to each other. Following the experiments, the precise probe separation was determined by inserting steel pins in each set of holes. The pins were then withdrawn from the holes with double-sided tape stuck to a card, which ensured that the original orientation of the pins was maintained. A line was then traced along both sides of each pin on a second card. The distance between the heater and upper and lower thermistors (probe

separation) was then measured at a point corresponding to the depth of the thermistors with a calibrated eyepiece.

#### Measurement of the diameter at cambium and heartwood

The salmon-pink heartwood of *E. grandis* is easily distinguished from the cream-coloured sapwood. The distinction is enhanced by the application of methyl orange stain to a cross section of the stem. This results in a crimson-coloured heartwood through reaction with the acidic tyloses, and a neutral orange colour in the sapwood (Bamber and Fukazawa 1985). The diameter of the outer limit of the sapwood (referred to as the diameter at cambium) and the diameter of the heartwood, were then taken as the average of four measurements on the section of the stem containing the HPV probes.

#### Sensitivity analyses

These analyses were conducted through a mathematical simulation of the effect of errors in the measurement of the following parameters: probe separation, wound size, wood density, and moisture fraction. These were selected as they were felt to be the most likely sources of error in the estimation of sap flow. The effect of increasingly serious errors in each of the above variables was calculated for a typical data set from one of the 3-year-old trees.

## Results

#### Verification on young *E. grandis* trees

Four cut-tree experiments were conducted on trees between 3 and 4 years of age (Table 1). Sap flow estimated with the HPV technique corresponded well with the measured uptake of water from the bucket (Fig. 1). This good correspondence was, however, very dependent on the accurate determination of wound size. At worst the cumulative uptake recorded by the two methods differed by 12.7% and at best 2.4% with an average of 4.0% for the four trees.

The moisture fraction (Table 2) varied between sample trees, ranging from 1.26 to 1.96, with a mean of 1.64. The wood density was more constant, ranging from 0.37 to 0.40, with a mean of 0.38. The measured wound widths varied considerably considering that both the drilling technique and the interval between probe implantation and measurement were relatively constant between the sample trees. This may be due to some variation in the alignment of the probe holes in the vertical plane, rather than solely to variations in the biological response to wounding.

It was of some concern that four probes may not cover variation in sap flux in the sapwood adequately. However, the success of the four cut-tree experiments conducted on young *E. grandis* trees indicates that four probes, each with a single thermistor, were sufficient for the young trees. There

are also physical limitations on how many probes one can insert in the stem of a small tree without either causing serious disturbance to sap flow or implanting the probes in undesirable positions.

#### Sixteen-year-old *E. grandis*

On completion of this study, a total of 238.8 L had been taken up from the bucket compared with a HPV estimate of 266.5 L, a difference of only 11.6%. The daily course of transpiration (Fig. 2) shows that the HPV estimates were generally higher than the cut-tree estimates, especially during the course of the morning, while the midday results compare more favourably. The fluctuations in the HPV estimates before 08:00 are unusual and difficult to explain. Difficulties were experienced in adding large quantities of water ( $40 \text{ L} \cdot \text{h}^{-1}$ ) to the flexible bucket in a short space of time, resulting in the fluctuations in the cut-tree estimate of water use over midday. The correspondence between the two measures of water use was nonetheless remarkably good considering that only eight probes were used on such a large tree. This corresponds to a sampling intensity of one probe per  $46.5 \text{ cm}^2$  sapwood, while four probes in a small tree (13 cm in diameter with a 3.0 cm thick sapwood) corresponds to one probe per  $23.6 \text{ cm}^2$ . The sampling intensity in the smaller trees was thus nearly twice that of the larger tree. This is a potential source of error that is best dealt with by sampling as intensively as practical.

#### Sensitivity analyses

The estimate of sap flow is sensitive to errors in the measurement of wound size, probe separation, and moisture fraction, but is not very sensitive to errors in the estimation of wood density (Table 3). The response to errors in wound size and probe separation is nonlinear, errors being relatively more serious when they are large than when they are small. For example, a 10% error in wound size results in an 18.0% error in flux, while a 50% error in wound size results in a 120.3% error in flux. Overestimates also tend to result in more serious errors than underestimates of the same magnitude.

#### Discussion

Physically and anatomically, *E. grandis* is an ideal species for HPV work. The bark is generally thin (rarely thicker than 25 mm), and the thin sapwood (usually no thicker than 30 mm) means that long probes are not required. The absence of resin facilitates the insertion and removal of the delicate probes. Anatomically, *E. grandis* is a diffuse porous hardwood with approximately eight vessels per square millimetre and vessel diameters ranging from 0.081 to 0.160 mm (Malan 1988). Growth rings are either only vaguely evident or absent altogether (Malan 1988). The estimation of wound size from excised sections of the sapwood is relatively simple provided about 5 days have passed to allow adequate development of tyloses.

Initial attempts at verifying the accuracy of the HPV apparatus revealed very poor correspondence between the cut-tree and the HPV estimates of water use. A gradual decline in water uptake was evident through the course of the morning despite increased levels of photosynthetically active radiation and VPD. Green and Clothier (1988) reported a similar decrease in the uptake of water due to the blockage of vessels, which was remedied by recutting the stem. Recutting *E. grandis* resulted in an immediate

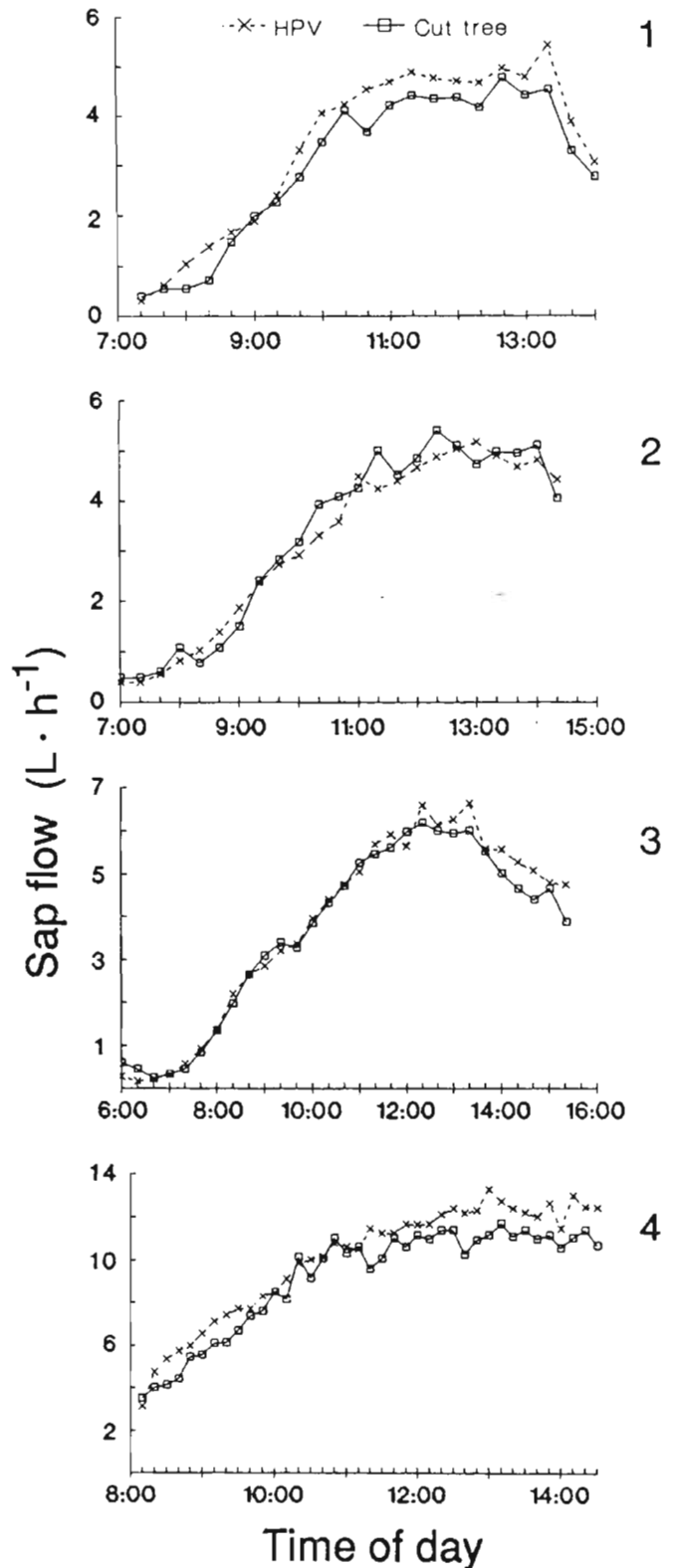


FIG. 1. A comparison of HPV and cut-tree estimates of sap flow through the course of a day conducted on four 3-year-old *E. grandis* trees. The HPV values were corrected for the wound associated with the implantation of the thermistor and heater probes.

increase in water uptake, which indicated that the vessels had been blocked, though the HPV technique still continued to seriously underestimate actual water uptake.

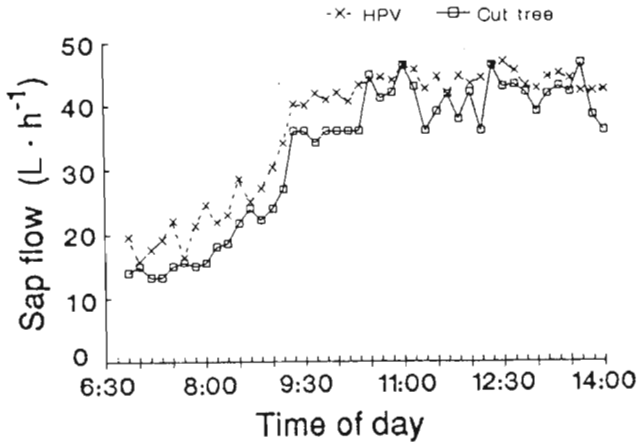


FIG. 2. A comparison of HPV and cut-tree estimates of sap flow through the course of a day conducted on a 16-year-old *E. grandis* tree. The HPV values were corrected for the wound associated with the implantation of the thermistor and heater probes.

TABLE 2. The moisture fraction, wood densities, and wound widths (mean  $\pm$  SD) of the five *E. grandis* trees used in the verification experiments

Tree No.	Age (years)	Moisture fraction, $m_c$	Wood density, $\rho_b$ ( $\text{g} \cdot \text{cm}^{-3}$ )	Wound width (mm)
1	3	$1.26 \pm 0.083$	$0.38 \pm 0.013$	$2.74 \pm 0.388$
2	3	$1.96 \pm 0.065$	$0.37 \pm 0.006$	$2.33 \pm 0.372$
3	3	$1.73 \pm 0.089$	$0.38 \pm 0.020$	$2.97 \pm 0.318$
4	3	$1.73 \pm 0.048$	$0.39 \pm 0.006$	$2.81 \pm 0.403$
5	16	$1.52 \pm 0.091$	$0.40 \pm 0.012$	$3.23 \pm 0.237$

In searching for reasons for this lack of correspondence, it was found that the sapwood was evenly stained by the dye added to the water, except for the radial band, where the flow of water was interrupted by the HPV probes. The width of this band ( $\pm 2.74$  mm) was invariably wider than the 1.85-mm drill bit used in the implantation of the probes. When the width of this band (rather than the drill size) was used as a measure of wound size, a good correspondence between the two estimates of water use resulted, implicating this as the major source of error. It was later found that the unstained band corresponded with tyloses deposited in the vessels, a process that takes about 5 days to occur. Hence wound size estimates in intact trees can be made from measurements of the band of tyloses on excised sections of the sapwood. The sensitivity analyses also indicate that it is important to measure this wound size accurately, particularly since the experimental data show that wound size was on average 48% greater than the size of the drill used.

The response to wounding depends on season (Lopushinsky 1986), phenological state (Shigo and Hillis 1973, as cited in Swanson 1983), and increases with time after probe implantation (Swanson and Whitfield 1981). These factors are probably largely responsible for the variation observed between the sample trees. This, together with the implications of a serious error in wound size, indicates that it is necessary to measure wound size with each implantation of probes. Unfortunately the simpler system employed by Swanson and Whitfield (1981) in adding 0.4 mm to the drill size would not suffice in *E. grandis*.

TABLE 3. The sensitivity of calculated flux to errors of varying magnitude in the measurement of selected variables

Error in measured variable (%)	Resultant error in sap flow (%)			
	Wound size	Probe separation	Moisture fraction	Wood density
10	18.0	18.7	8.3	10.0
20	40.3	39.0	16.7	20.0
-20	-29.7	-32.6	-16.7	-20.0
50	120.3	109.6	41.9	50.0

The sensitivity to errors in the measurement of probe separation indicates that it is also necessary to take particular care over these measurements. Although the use of steel pins was apparently adequate, direct measurements of excised sections of the stem containing the probe insertion holes would be more reliable (if destructive sampling is acceptable). This method would also be much easier to apply in trees where probes were deeply implanted or in trees that exude resin.

A likely source of error is in the measurement of the moisture fraction, particularly if care is not taken to prevent the evaporation of water from the wood sample. Although this is a likely source of error, its potential magnitude is not as serious as that which can be made in the estimation of the wound size.

There are differences in the published literature regarding the sequence of calculations followed in converting heat pulse times to sap flow. This paper follows the method and terminology of Marshall (1958) and Swanson (1983), where the correction for wounding (Swanson and Whitfield 1981) is applied to the HPV. However, Edwards and Warwick (1984) apply the wound correction to what they call sap velocity (equivalent to sap flux), and Green and Clothier (1988) apply it to sap flux directly. Since the correction is a function of velocity squared, this can result in an underestimation of sap flow by as much as 20%. Errors are particularly serious at high sap flow rates and large wound sizes. The approaches of Edwards and Warwick (1984) and Green and Clothier (1988) do, however, give the same result as Swanson (1983), provided the wound corrections are applied to the HPV rather than to sap flux.

Sap flow has been measured in stems of trees using as few as one set of probes (Doley and Grieve 1966), to as many as 16 (Swanson 1983). Most commonly either four (Edwards and Warwick 1984; Green and Clothier 1988) or six probes (Cohen *et al.* 1981) are used per stem. Few have thoroughly examined how many are actually required and where they should be placed in the sapwood for accurate estimates. Clearly, given enough insight into the variation in sap flux with depth in the sapwood along various radii, it is theoretically possible to derive accurate estimates of sap flow with a single strategically placed sensor. However, since this information is generally not available beforehand, it is important to use enough probes to cover likely variation in the sapwood.

Swanson (1983) felt that 8 to 12 probes should be adequate in measuring sap flow in the stems of unstressed trees, but this should be increased to one per square centimetre (sapwood) for stressed trees. The results presented for *E. grandis* suggest that four probes are adequate for young



*E. grandis* trees (one probe for 25 cm<sup>2</sup> sapwood), while older trees require at least eight probes per stem (one probe per 45 cm<sup>2</sup>). These sampling intensities coincide with those of Swanson (1983). It would, however, be advisable to determine the precise sampling requirements prior to fieldwork, using a technique such as outlined by Dye *et al.* (1991), since these may well differ between species and perhaps even growing conditions.

### Conclusion

This study has shown that the HPV technique is particularly suitable in the study of water use from the diffuse porous hardwood *E. grandis*. The technique accurately reflected water uptake in both age-classes. The method does, however, demand particularly accurate wound size and probe separation estimates.

For the successful field application of the apparatus in *E. grandis*, it is recommended that (i) at least 5 days should pass between probe implantation and sampling to allow adequate time for tylosis development (this facilitates wound size measurement); (ii) particular care be taken over the measurement of wound size and probe separation; (iii) at least eight probes are used on trees larger than about 20 cm in diameter, while four probes are adequate for smaller trees.

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[3]

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

B.W. Olbrich<sup>\*a</sup>, D. Le Roux<sup>b</sup>, A.G. Poulter<sup>a</sup>, W.J. Bond<sup>b</sup>, W.D. Stock<sup>b</sup>

<sup>a</sup>*Division of Forest Science and Technology, Council for Scientific and Industrial Research,  
Private Bag X11227, Nelspruit, 1200, South Africa*

<sup>b</sup>*Department of Botany, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa*

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hat 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  $^{13}\text{C}$  to  $^{12}\text{C}$  (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,  $^{13}\text{C}$ , and light,  $^{12}\text{C}$ , 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,  $^{13}\text{C}$ . Conversely, plants with a high water use efficiency should have relatively more of the heavier isotope,  $^{13}\text{C}$ , 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

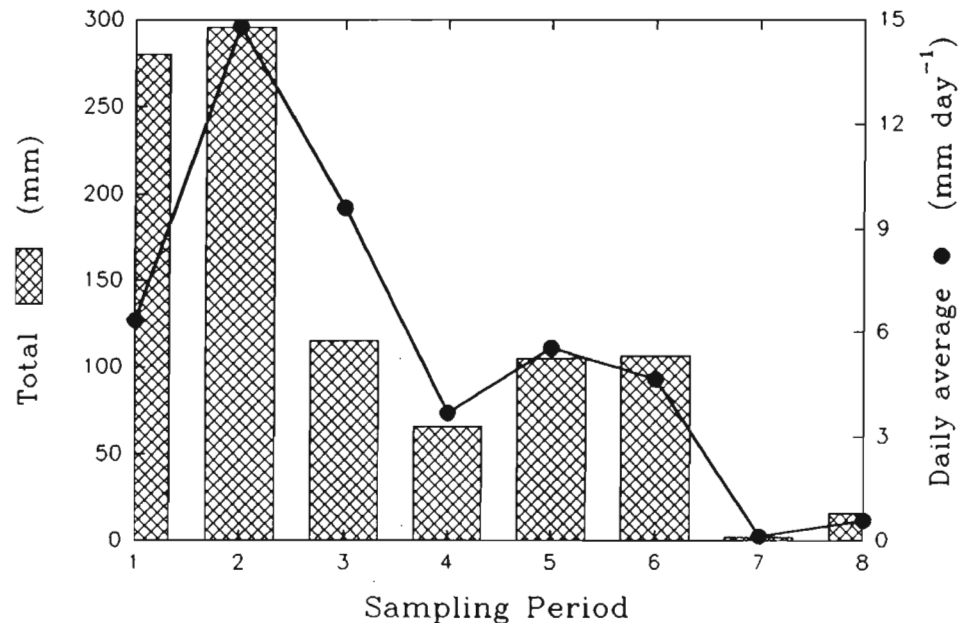


Fig. 1. The total (bars) and average daily (solid line) rainfall during each sample period.

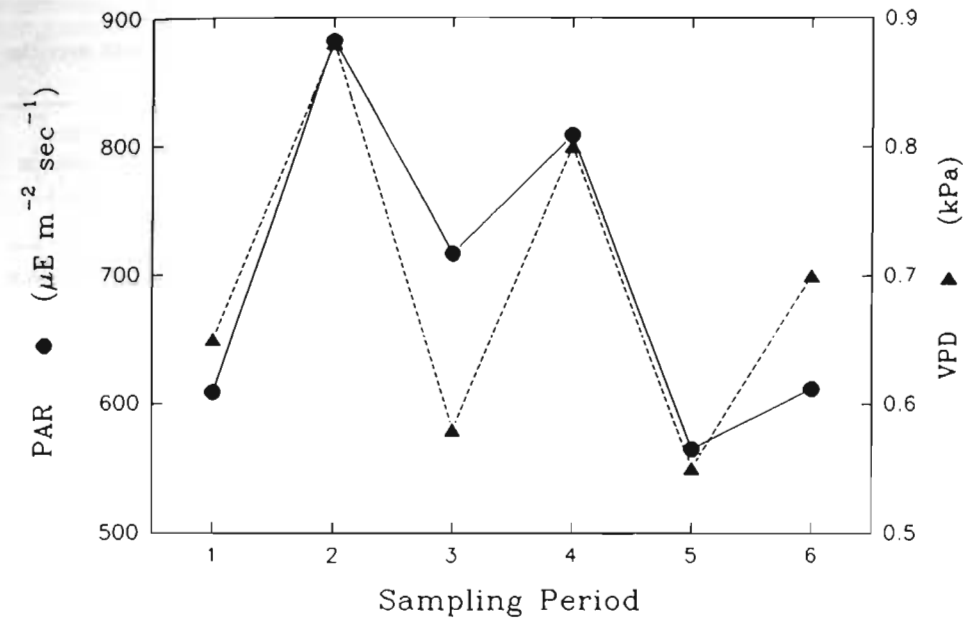


Fig. 2. The daily average daytime photosynthetically active radiation (PAR), and vapour pressure deficit (VPD) during each of the six sample periods.

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 <sup>2</sup> )	Leaf-area: sapwood-area ratio (m <sup>2</sup> cm <sup>-2</sup> )
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 <sup>3</sup> )
		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

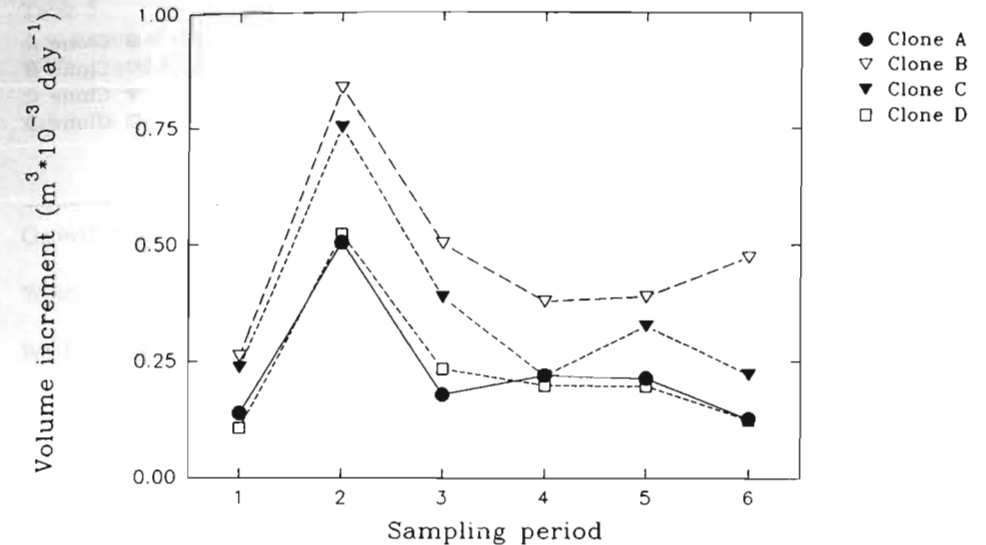


Fig. 3. The clonal-average daily volume increments measured in m<sup>3</sup> day<sup>-1</sup>.

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 (1 tree<sup>-1</sup> day<sup>-1</sup>) for each of 11 sample trees

Tree	Clone	Regression	No. days sampled	R <sup>2</sup>
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 ( $y$ )

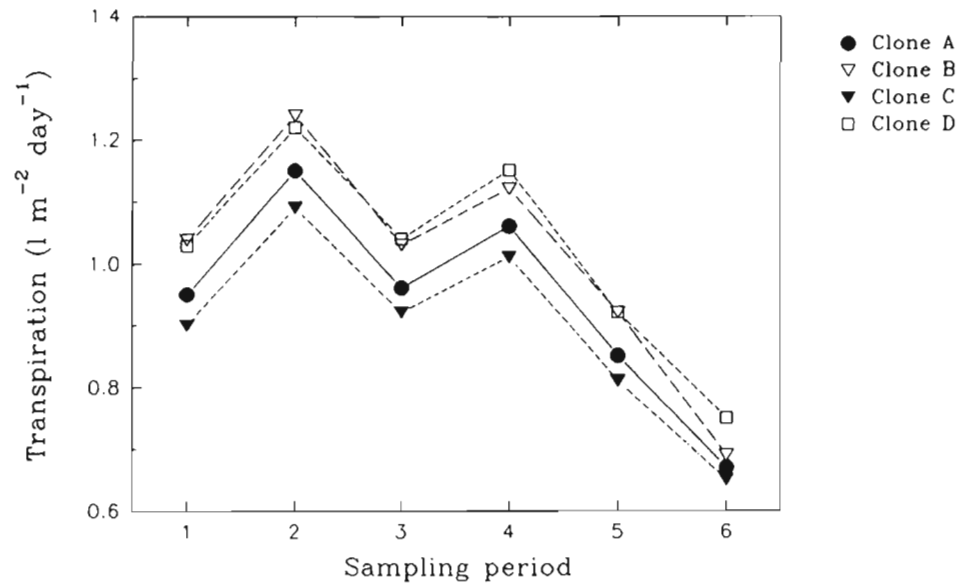


Fig. 4. The clonal-average daily water use per square metre of foliage.

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  $\delta^{13}C$  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
$\delta^{13}C$ (‰)	$-23.3 \pm 0.17$ 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  $m^3$  while growth varied from  $1.43 \times 10^{-2}$  to  $7.14 \times 10^{-2} m^3$  over the study period. The clonal-average  $WUE_{sl}$  varied from 0.066 to 0.017  $m^3$  wood produced per  $m^3$  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  $m^3 m^{-3}$ . The fourth clone, clone D, had the lowest water use efficiency of 0.007  $m^3 m^{-3}$ . 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



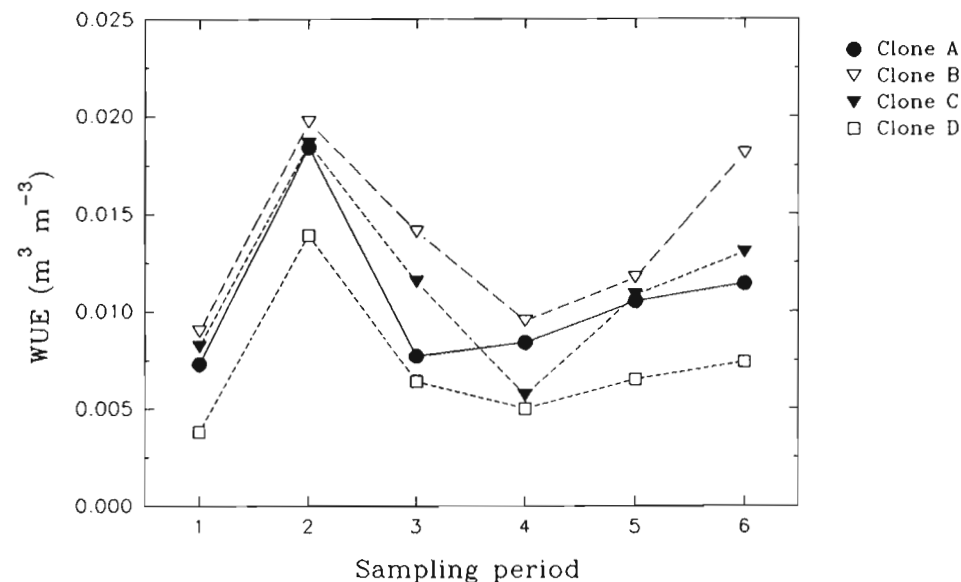


Fig. 5. The clonal-average water use efficiency in  $\text{m}^3$  stem volume increase per  $\text{m}^3$  water transpired, recorded for each of the six sampling periods.

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  $\text{WUE}_{\text{av}}$  was particularly variable in clones B and C (Table 5, Fig. 6). The large variation in  $\text{WUE}_{\text{av}}$  recorded in clone B was primarily due to the low  $\text{WUE}_{\text{av}}$  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  $-29.66\text{‰}$ , which is typical for  $\text{C}_3$  plants (O'Leary, 1981). Significant clonal differences were found in the average  $\delta^{13}\text{C}$  of the leaves sampled from the canopy (Table

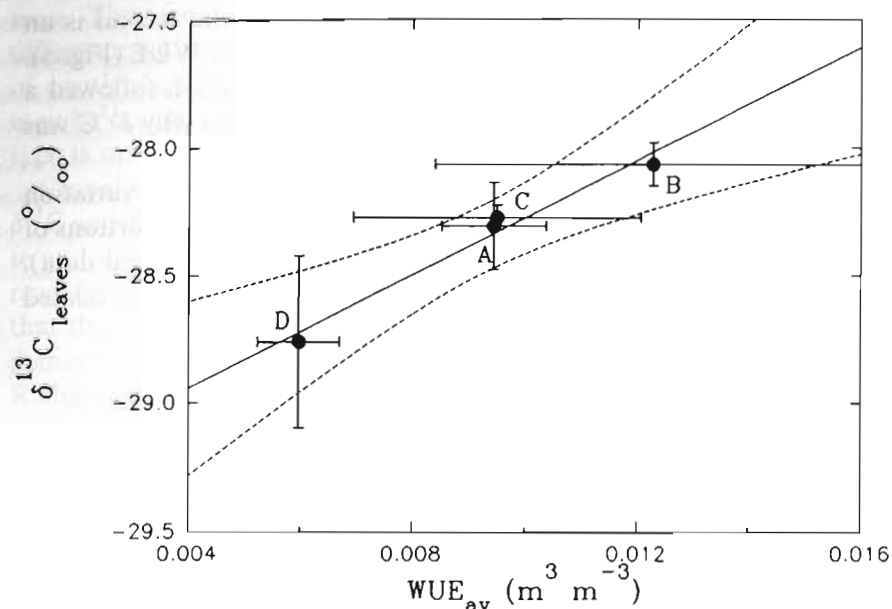


Fig. 6. The relationship between the average water use efficiency ( $\text{WUE}_{\text{av}}$ ) of the four clones and the mean  $\delta^{13}\text{C}$  level of leaves from the three canopy positions sampled (mature leaves from the northern and southern side of the canopy, and young foliage). The error bars represent 1 standard deviation of the mean and the lines represent the regression between clonal-mean  $\text{WUE}_{\text{av}}$  and  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values than the other three clones.

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

The difference in  $\text{WUE}_{\text{sl}}$  and  $\text{WUE}_{\text{av}}$  is that the first measure integrates

Table 6  
Clonal mean  $\delta^{13}\text{C}$  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  $\text{WUE}_{\text{av}}$ .

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  $\text{WUE}_{\text{av}}$ .

### 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 ( $\text{WUE}_{\text{cl}}$ ) 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  $\text{WUE}_{\text{av}}$  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  $\text{WUE}_{\text{av}}$  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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