GROWTH, WATER USE EFFICIENCY AND STABLE CARBON ISOTOPES IN COMMERCIAL CLONES OF *EUCALYPTUS*

DEBBIE JEANETTE LE ROUX

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ABSTRACT

The expansion of *Eucalyptus* plantations to supply timber for an increasing population in South Africa will result in a great reduction in the country's run-off water. If *Eucalyptus* continues to be the source of timber in South Africa, the selection of more water use efficient species for planting in existing and new areas has to be implemented. An understanding of the physiological factors ruling growth and water use efficiency in *Eucalyptus* is needed to develop selection criteria for improved water use efficiency and harvestable stem production under a limited water supply.

This study investigated the effects of soil moisture availability on the growth and water use efficiency of 6 commercial clones of *Eucalyptus* commonly grown in South Africa with the aim of determining the following:

(i) The extent of clonal variation in growth, dry mass allocation patterns, water use efficiency and the water cost of wood production at 16 months after planting.

(ii) The influence of plant physiological traits such as patterns of dry mass allocation, canopy leaf area, leaf canopy density, specific leaf area, foliar nitrogen concentration and instantaneous rates of photosynthesis and transpiration, on growth, water use efficiency and the water cost of wood production.

(iii) The complications associated with sampling for stable carbon isotope ratios ($\delta^{13}C$) within a eucalypt canopy and the potential use of $\delta^{13}C$ in plant tissues as a tool for ranking clonal water use efficiencies.

The growth of eucalypt clones was affected strongly by soil water availability. Under low soil moisture availability growth was reduced in proportion to the reduction in available soil water but an increase in water use efficiency was found. Clones did not differ in the total accumulation of dry mass but significant differences were found in the relative allocation of dry mass to roots, main stems, branches and leaves. Significant differences were found in clonal water use efficiencies and the rankings were preserved under both soil water availabilities. The water cost of wood production differed significantly amongst clones and this variation was influenced largely by the clonal variation in the dry mass allocation to main stems.

Growth was not significantly correlated with photosynthetic capacity per unit leaf area but was enhanced through high allocation to roots resulting in high root:shoot, root:leaf area ratios, and large canopy leaf areas with low specific leaf areas. Increased water use efficiency was associated with dense leaf canopies and high foliar nitrogen concentrations per unit area. A decrease in the water cost of wood production was found with an increase in foliar nitrogen per unit area and an increase in the allocation of dry mass to above ground plant parts relative to roots.

$\delta^{13}C$ values were found to vary between tissues and positions in 4 year old eucalypt canopies and amongst leaf organic pools (lipids, starch, ethanol soluble carbohydrates and leaf crude wall fibre). In 16 month old eucalypt trees, the best estimate of water use efficiency was derived from the $\delta^{13}C$ value of the leaf crude wall fibre. Despite the significant correlation between $\delta^{13}C$ and water use efficiency found in this study, a large proportion of variation in water use efficiency is not explained by variation in $\delta^{13}C$ implying that the relationship is more complicated in trees than for annual crops. However clonal variation in $\delta^{13}C$ was associated with clonal variation in water use efficiency at 16 months. The ranking of clonal growth performances under conditions of water shortage in 4/5 year old trees were maintained.

This thesis presents the integration of a physiological understanding and the practical implications for the potential improvement and early selection for water use efficiency and harvestable stem production in commercial clones of *Eucalyptus* commonly grown in South Africa.

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CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Commercial Forestry in South Africa

Commercial afforestation with eucalyptus and pines can be viewed as an invaluable replacement source of timber to the existing indigenous forests both in Southern Africa and the rest of Sub-Saharan Africa (Schönau, 1991). Out of a total land area of approximately 112 million ha, approximately 1.1% or 1 197 850 ha comprises commercial plantations in South Africa, 50% of it occurring in the Transvaal, 40% in Natal and the other 10% in the Cape Province (Rusk et al., 1991).

Planting with eucalypt species (predominantly *Eucalyptus grandis* Hill ex Maiden) in South Africa began at the beginning of the twentieth century and presently makes up approximately 40% (476 770 ha) of the country's total commercial plantations. Most of the plantations occur in the summer rainfall area, along the east coast and eastern interior where the rainfall is in excess of 750 mm per annum (Rusk et al., 1991).

Eucalyptus grandis (Hill ex Maiden) is the most widely planted and successful hardwood covering 80% of the region and about 85% of its roundwood produced in this country is consumed by the mining and pulp industries, each responsible for about an equal share (Malan, 1991). With the introduction of the genetic improvement programme in 1983, many other species such as *E. nitens*, *E. elata*, *E. fastigata*, *E. macarthurii* and *E. camaldulensis* have been planted in provenance trials for the improvement of growth rate, stem form and wood quality (Stanger, 1991).

1.2 The problem

The impact of commercial afforestation on the country's water supply was estimated to be approximately 1284 million m^3 consumption per annum during 1980 (Bosch and von Gadow, 1990). The long term demand for pulp wood remains firm and on the increase due to the growing population size and future improvement in living standards of a large section of the population in South Africa. It has been estimated that timber supply should increase by approximately 80% to meet the rising demand over the next 20 years (Bosch and von Gadow, 1990). If timber supply is to match demand, an 80% increase in forested area should result in a 32% increase in total water use by commercial plantations under conditions of better forestry management (Bosch and von Gadow, 1990).

Afforestation with *Eucalyptus* has proven to be attractive due to their relatively fast growth rates and good wood properties and there is an increasing switch from planting with pine to eucalypt species both on a global scale and in South Africa (Schönau, 1991, Turnbull, 1991). Since the lifting of international sanctions on South Africa, the opportunities for the sale of pulpwood derived from eucalypt species as a replacement source of timber to indigenous forests, have increased substantially (Keyworth, 1993). However the major trade-off associated with afforestation with eucalypt species has been their higher consumption of water relative to indigenous or other commercial forest species (van Lill et al., 1980, van Wyk, 1987, Bosch and Smith, 1989). Bosch and von Gadow (1990) have indicated that the mean annual reduction in stream flow associated with the planting of *Eucalyptus* in catchment areas was approximately 800% higher than that of pines during the first seven years of growth (Figure 1.1).

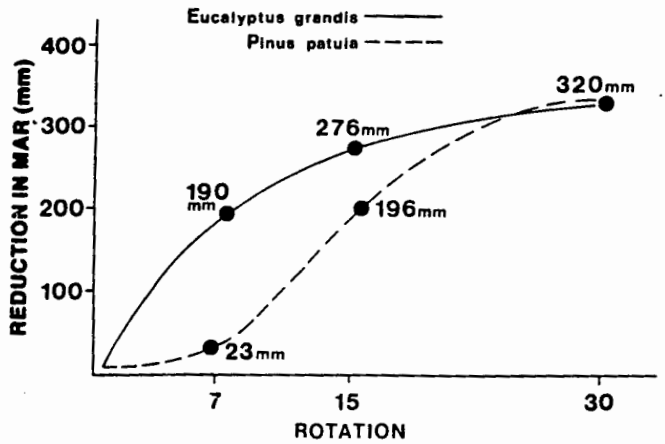


Figure 1.1 The relationship between mean annual runoff reduction, as a result of afforestation, and the clearfelling rotation for two commercial forest species *Eucalyptus grandis* and *Pinus patula* (from Bosch and von Gadow, 1990).

Since South Africa is a water-limited country with an average rainfall of 475 mm year^{-1} (Jewitt and Schulze, 1991), the afforestation with *Eucalyptus* in existing catchment areas is in conflict with other water requiring practices (Bosch and von Gadow, 1990). However the water costs of afforestation with eucalypt species may be reduced by selecting trees that use water more efficiently without compromising wood production.

1.3 The approach

Up until now, selection for increased yield and wood quality in commercial forestry has been successfully based on the method of screening for these traits in the field and promoting them through vegetative propagation. However, major losses to the forestry industry due to tree mortality during the recent drought (1991/1992) experienced in South Africa, has evoked greater concern in the area of improving silvicultural practices and selecting for water use efficient species that would not only survive but continue to be productive under restricted water conditions.

To date estimates of water use of individual species in mature commercial forest stands have been based on canopy conductance models and sap flow estimates using the heat pulse velocity technique (Olbrich 1991, Dye and Olbrich, 1993). No attempts have been made to screen for water use efficiency at the juvenile stage for possible early selection of suitable genotypes for the planting in existing and marginal plantation areas.

Water use efficiency (unit biomass produced per unit water used) has not been a criterion in tree breeding in this country, or anywhere else in the world, purely due to the lack of suitable techniques available to screen for this trait. Several physiological traits such as differences in leaf water potential, leaf diffusion resistance, soil water extraction capacities (Blum 1974a), cell wall elasticity (Blum 1974b), rooting patterns, leaf hairiness and stomatal frequency (Burton et al. 1977) have been associated with increased drought tolerance and water use efficiency in crop plants. However increases in water use efficiency may not always be associated with drought tolerance in natural environments since drought adaptive traits may enable a plant to withstand water shortages but with low biomass accumulation (Hunt, 1962, Barnes, 1983).

Recently, it has been suggested and empirically shown that the relative abundance of stable carbon isotopes, ^{13}C and ^{12}C, in plant tissues is related to dry mass accumulation and water use efficiency in crop genotypes (Farquhar et al., 1982, Farquhar and Richards, 1984). Genetic variation in water use efficiency and stable carbon isotope ratios have been found in tomato, peanut and potato cultivars (Hubick et al., 1986, Martin and Thorstenson, 1988, Vos and Groenwold, 1989), which suggests the potential use of stable carbon isotopes as a

tool in the early screening for water use efficiency under field conditions. Furthermore the relative ratios of stable carbon isotopes may provide a practical method for ranking water use efficiencies which will aid in the process of identifying genes associated with, and the mechanisms contributing to, this trait.

At this stage genetic studies associated with the selection for drought tolerance and water use efficiency in agricultural crops and trees are still in their infancy. However with the changing global climate it is possible that there may be an increase in the frequency of drought cycles occurring in South Africa in the future (Schulze, 1991). Therefore there is an urgent need for co-ordinated research between forestry managers, plant physiologists and geneticists with the aim of improving crop yield per hectare under water limited conditions.

This thesis presents an ecophysiological study of six commercial clones of *Eucalyptus* commonly grown in South Africa. The aims of the study were (i) to determine the effect of soil moisture availability on dry mass accumulation and water use efficiency in eucalypt genotypes (Chapters 2 and 3), (ii) to determine the extent of genetic variation in relative growth rates, patterns of dry mass allocation to harvestable stem wood and water use efficiency under different soil moisture availabilities (Chapters 2 and 3), (iii) to provide a better understanding of the physiological processes controlling growth and water use efficiency of eucalypts under different soil moisture availabilities (chapters 2 and 3), and (iv) to validate the potential use of stable carbon isotopes as a selection tool for assessing water use efficiency in *Eucalyptus* (chapters 4 and 5). An integration of the physiological findings and practical implications of this study (Chapter 6) should provide useful information concerning the criteria which need consideration when selecting for higher yield and water use efficiency.

CHAPTER TWO

GROWTH PHYSIOLOGY OF COMMERCIAL CLONES OF *EUCALYPTUS* AS AFFECTED BY WATER AVAILABILITY

2.1 INTRODUCTION

Plant growth or productivity can be defined as the accumulation of biomass which is fundamentally dependent on the acquisition of carbon, mineral nutrients and water and the conversion into more complex molecules using the sun's radiant energy. Comparisons made on the accumulation of biomass between plant individuals become equitable when using mean relative growth rate (RGR), where the biomass increment is calculated proportionally to the existing biomass over time (Hunt, 1978).

Innate differences in relative growth rates have been found amongst both crop and wild species and clonal genotypes (Grime and Hunt, 1975, Gifford and Thorne, 1984, Hunt and Lloyd, 1987, Evans, 1991). This variation has been attributed to habitat-related variation in abiotic factors, such as temperature, water, light and nutrients or by biotic factors such as competition, diseases or grazing pressure (Blackman and Wilson, 1951, Grime and Hunt, 1975, Hunt and Lloyd, 1987). However large interspecific variation in RGR exists even when grown under identical, near-optimal conditions free of interference from other organisms (Poorter, 1989). It is the latter condition that this chapter focuses on with the aim of determining factors controlling the growth physiology of eucalypt genotypes under partially controlled conditions in the field.

Several hypothetical approaches and empirical plant growth analyses have emerged in the literature during the past decade (Mooney, 1972, Thornley and Reynolds, 1982, Bloom et al., 1985, Konings, 1989, Ceulemans, 1989, Poorter, 1989, Farrar, 1989, Lambers et al. 1989, Mooney and Winner, 1991) most of which parallel the central concept that optimal growth is achieved through changes in biomass partitioning to functional tissues (i.e. leaves, stems and roots). At the same time the spatial arrangement of functional tissues is such that the maximum net carbon gain under a given range of external resources i.e. mineral nutrients, water and light is maximised. Essentially the growth of new leaves and supporting stems have a positive feedback upon the production process and new roots have a positive feedback upon the plant nutrient and water status (Schulze et al.,1983). Allocation to leaf, stem and root tissues are adjusted so that all resources equally limit growth (Bloom et al. 1985).

Although plant growth is dependent on the assimilation of carbon through photosynthesis, many studies on herbaceous species have shown that RGR does not correlate with photosynthetic activity per unit area (Gifford and Thorne, 1984, Poorter, 1989, Konings, 1989). Instead RGR is determined by the net assimilation rate, (NAR) or leaf area ratio (LAR) (see Poorter, 1989 for review). NAR describes the increase in plant weight per unit leaf area, which is the balance between carbon gained through photosynthesis and whole plant respiratory losses expressed on a leaf area basis. LAR describes the product of the leaf weight ratio (LWR, the fraction of material invested in the leaves), and the specific leaf area (SLA, the leaf area per unit leaf weight). These approaches emphasize the importance of considering the total amount of carbon assimilated by a canopy rather than the net photosynthetic activity at the level of the leaf.

Konings (1989) reported that in a wide range of herbaceous species nutrient and water availability had a direct effect on biomass allocation to optimize RGR. In a nutrient poor or dry habitat, plants will allocate a greater proportion of biomass to roots for the aquisition of nutrients and water. Consequently they will be characterised by large root:shoot ratios, small leaves with high nitrogen concentrations and rates of photosynthesis per unit area and low specific leaf areas, in which case RGR is achieved through a high NAR (Konings, 1989). On the other hand, in an environment of higher nutrient and water availability, a greater proportion of biomass is allocated to leaves and stems resulting in low root:shoot ratios, large canopy leaf areas, leaves with larger SLA with lower nitrogen concentrations and rates of photosynthesis per unit area and high RGR is achieved through high LAR (Konings, 1989). NAR and LAR are generally negatively correlated with each other and differences in RGR are often counteracted by a species having high NAR and low LAR or vice versa.

Studies of growth strategies of tree species are less common than those of herbaceous species, however it has been established that forest productivity is closely related to the leaf area available to intercept solar radiation (Waring and Schlesinger, 1985, Landsberg, 1986, Sands et al., 1992). Recent studies on *Eucalyptus* species showed that nutrient and water availability are the major determining factors of biomass accumulation and allocation (Mooney et al., 1978, Sheriff and Nambiar, 1991, Pereira et al., 1993). Mooney et al. (1978) showed that eucalypt species growing in arid habitats in Australia exhibited higher root:shoot and root:leaf area ratios, higher nitrogen content per unit leaf area, higher

photosynthetic rates per unit leaf area and higher NAR than species growing in humid regions. In constrast Pereira et al. (1993) showed that irrigation and fertilization of *E. globulus* seedlings in the field in Portugal did not affect photosynthetic capacity and leaf nitrogen concentrations of leaves, but caused a significant increase in canopy leaf area and biomass production. For seedlings of the same species and *E. camaladulensis* in a pot experiment in Australia, Sheriff (1992) reported increases in growth, foliage area and foliar nitrogen concentrations with increased nitrogen supply. An experiment carried out on *E. grandis* seedlings under varying nitrogen conditions indicated that under low nitrogen conditions growth was mediated through allocation to roots and leaf photosynthetic rates whereas high nitrogen conditions resulted in growth being mediated through changes in SLA (Sands et al., 1992).

Research results outlined above show that induced changes in allocation, SLA and photosynthetic capacity are the dominant processes controlling RGR in tree seedlings under varying nutrient and water availabilities. In South Africa, the availability of water is the major factor limiting growth of commercial clones of *Eucalyptus* (Boden, 1988). The ability to screen for criteria that underlie RGR of various genotypes under different moisture availabilities would contribute enormously to the selection and planting of the appropriate genotypes in areas of a known moisture regime.

It is acknowledged that productivity in terms of harvestable stem biomass of eucalypt clones is of primary importance to the commercial forester. Since it is only a proportion of assimilated carbon that is allocated to harvestable wood (approximately 20%) which is subject to change under varying environmental conditions, the determination of factors controlling RGR would be only the first step in the process of estimating forest productivity and wood production (Waring and Schlesinger, 1985). Agronomical studies have shown that increases in economic yield potential (e.g. grains, tubers, sugar storage stem) have been achieved mainly through manipulation of the partitioning of photosynthetic products between harvestable and non-harvestable biomass and not through increases in total crop biomass or RGR (Gifford and Thorne, 1984). In trees, harvestable stem wood functions mainly for storage of carbon reserves and the conduction of water, nutrients and carbohydrates between leaves and roots. It is expected that an increase in RGR brought about by greater leaf

production would have an effect on stem diameter growth since new foliage demands new sapwood (Waring and Schlesinger, 1985). Because carbon allocation to stem wood has low priority in trees, stem wood biomass expressed per unit foliage area has been suggested to serve as a "growth efficiency" index in trees. High values indicate a greater balance in the distribution of carbohydrates whereas low values suggest limitations in storage reserves and in the ability of the tree to produce various protective chemicals (Waring and Schlesinger, 1985). However little is known about the factors controlling the allocation to storage biomass in the stem relative to other plant functional attributes in trees, and it is an area of research that needs further exploration.

As yet, little is known about the growth physiology of clonal eucalypts in South Africa and the first step towards improving selection for growth performance is to understand the processes controlling RGR and patterns of dry mass allocation under different soil moisture regimes. With this objective, this study was designed to answer the following specific questions:

1. How does soil moisture availability affect carbon allocation, SLA and photosynthetic activity in eucalypt genotypes commonly grown in South Africa ?
2. Which plant growth traits are most closely related to relative growth rates in these *Eucalyptus* clones ?
3. Is there clonal variation in RGR and dry mass allocation to harvestable wood among the various *Eucalyptus* clones. If so, do the phenotypic responses to soil moisture availability differ among the various clones ?
4. Which plant growth traits are most closely related to dry mass allocation to harvestable stem wood in the *Eucalyptus* clones being studied ?

2.2 METHODS

2.2.1 Study Site

The study site is located at 950 metre elevation at the D.R. de Wet Forestry Research Centre near the town of Sabie, eastern Transvaal, South Africa (25^{0}3'10"S, 30^{0}53'30"E) (Figure 2.1).

The climate is subtropical with infrequent frost. The mean annual precipitation is

approximately 1250 mm, with a mean annual minimum and maximum temperature of 6.9 and 26.2^{0}C respectively. The soils are red ferralitic clays of the Farningham Series of the Hutton form according to the South African binomial classification system (MacVicar et al. 1977).

Figure 2.1 Map showing location of D.R. de Wet Research Station* in the eastern Transvaal, South Africa.

2.2.2 Clonal material

Cuttings of six clones of *E. grandis* and hybrids with *E. camaldulensis* and *E. nitens* that are commonly grown in provenance trials in HL&H plantations in the Eastern Transvaal were used in the study. Clonal identities are provided in Table 2.1.

Table 2.1. List of clones used in the pot trial at Sabie. Clones were provided by HL&H Tree Breeding Centre. Throughout this thesis, clones are referred to by the clone number in the left-hand column.

Clone number	Specific names	HL & H clonal ID
1	grandis x camaldulensis	GXC 011/025
2	grandis	JDM 254/005
3	grandis	KFT 023/033
4	grandis	JDM 063/002
5	grandis x nitens	GXN 191/401
6	grandis x nitens	GXN 360/148

2.2.3 Experimental design

Ten replicates of each clone were planted in pots (220 *l* drums) in the field. Rainfall was excluded from the pots by "plastic lids" designed in such a manner to allow for the protrusion of the plant stem and leaf canopy (Plate 1). For practical reasons, the pots were sunk to approximately 60cm in the soil to reduce overheating of the soil inside the pots and to facilitate water application and the measurement of growth parameters.

2.2.4 Planting

Clonal cuttings were planted at 15 cm below the soil surface on 21 March 1991. 3:2:1 NPK fertilizer (100 g) and 2 l of water were added to each drum on planting. After two weeks, a 20% mortality was recorded. Replacement planting of dead individuals took place on 2 April 1991.

2.2.5 Measurement of soil moisture

Soil moisture depletion through root uptake was measured with a neutron probe (Troxler

Depth Moisture Gauge, model 3300, Troxler Laboratories, North Carolina, USA). Pots were fitted with 10 cm diameter, aluminium access tubes to facilitate the penetration of the soil with the probe (Plate 2). Neutron probe count ratios, calibrated against volumetric soil moisture in the pots (Appendix 1), were used as an index of soil moisture in the pots on a weekly basis. Initially one access tube was installed in the pot of a representative of each clone and treatment to provide an estimation of clonal water usage. Six months after planting large intraclonal differences in basal stem diameter and height were observed. This suggested that water usage among the individuals of the same clone was variable. An access tube was installed in each pot to provide a more accurate measure of water consumption of the individuals of each clone.

2.2.6 **Application of soil moisture**

Half of the individuals of each clone were subjected to a low (W_l) watering treatment and the remainder to a high (W_h) treatment where soil moisture in the drum was maintained at 60 and 80 *l* respectively, on a weekly basis. Data from the soil moisture release curves obtained at the end of the experiment (Appendix 2) indicate that the individuals from the high and low watering treatments operated in soil moisture ranges of 130-90% (80 to 50 *l*) and 95-70% (60 to 45 *l*) of field capacity respectively. Permanent wilting percentage defined at -1.5 MPa, was estimated to occur at approximately 68% of field capacity.

2.2.7 **Components of growth**

2.2.7.1 *Mean relative growth rates*

Mean relative growth rates were determined using the equation,

$$RGR = \ln W_2 - \ln W_1 / t_2 - t_1$$

where $\ln W_2$ and $\ln W_1$ are the natural logarithms of the total plant dry mass at the end and start of the growth period respectively, and t_2 and t_1 are the time in days when the plant dry mass was determined (Hunt, 1978). Initial plant dry mass did not vary significantly between clones ($F_{2,6} = 0.46$, $p > 0.05$) and a mean initial mass of 2.28 g was assumed as W_1 for all the clones.

2.2.7.2 *Dry mass accumulation*

Plants were harvested between 20 and 23 July 1992. The crowns were felled at 5 cm above

the soil surface and separated into leaves, side branches and main stems. Pots were dug out manually and the contents were passed through a sieve of 10 mm mesh size to separate roots from soil. Fresh mass was determined for each plant part and subsamples were taken for determination of fresh/dry mass ratios after drying at 80°C for 48 hours.

2.2.8 Correlates of growth

2.2.8.1 *Canopy leaf areas*

Canopy leaf areas were determined from leaf fresh mass. Leaf fresh mass was related to leaf area by regressing a sample of 100 leaves for each clone and treatment. Points along the regression line (leaf area vs fresh mass) were determined for 1, 5, 10, 15, 30 and 40 leaves.

2.2.8.2 *Specific leaf areas*

At the time of harvest, thirty healthy, fully expanded leaves were sampled from the northern side of the canopy. Individual leaf areas and dry weights were determined. Specific leaf area (SLA) was determined as leaf area/leaf weight ($m^2.kg^{-1}$).

2.2.8.3 *Leaf Area Index*

Canopy radii, r, were measured from the main stem to the tip of the longest branch extending horizontally to the canopy edge. Measurements were made on 29 July 1992. Canopy drip line area was calculated using the equation of a circle, πr^2. Canopy leaf area index was calculated as,

canopy leaf area (m^2)/canopy drip line area (m^2),

where canopy leaf area was derived in the manner described previously.

2.2.8.4 *Leaf weight ratio (LWR)*

Leaf weight ratio was determined as the dry weight of leaves present on trees at the time of harvesting expressed as a proportion of the total plant dry weight including roots (Poorter, 1989):

LWR = leaf dry weight / whole plant dry weight ($g.g^{-1}$)

2.2.8.5 *Leaf area ratio (LAR)*

Leaf area ratio was calculated as the product of SLA and LWR (Poorter, 1989):

$$LAR = SLA * LWR \ (m^{-2}.kg^{-1})$$

2.2.8.6 *Net assimilation rate (NAR)*

Net assimilation rate was calculated as the increase in whole plant weight per unit leaf area and per unit time (Poorter, 1989):

NAR = 1/LA * dW/dt, where LA equals whole plant leaf area and dW/dt is the change in whole plant dry mass on a daily basis over the growth period (g. m^{-2}. day^{-1})

2.2.8.7 *Root:shoot ratio*

Root:shoot ratios were calculated as the dry mass of roots expressed per unit dry mass of shoots (leaves, branches and main stem).

2.2.8.8 *Root:leaf area ratio*

Root:leaf area ratios were calculated as root dry mass expressed per unit leaf area to provide an index of the absorption capacity in relation to the transpiration surface which differs slightly from the more accurate use of root length or area described by Konings (1989).

2.2.9 Gas exchange measurements

On the morning that photosynthetic measurements were to be made the soil water content of pots were raised to the upper limit of the treatment level, i.e. 60 l in W_l and 80 l in W_h.

Leaf gaseous exchange measurements of CO_2 uptake at different photon flux densities (Q) were performed in the field using the PACsys 9900 flow control system (DDG design group, California, USA) and an ADC LCA2 infra red gas analyser (The Analytic Development Co. Ltd, Hoddeson, England) to determine light saturation (Q_l) on the 19 and 20 May 1992. Measurements were performed on five fully expanded leaves at mid height on the northern side of the canopy of a high watered individual of *Eucalyptus grandis* (JDM 254/005) in the pot trial. Chamber conditions were held constant at an air temperature of 27^{0}C and 19% relative humidity. Air entering the chamber was set at ambient CO_2 concentrations of 340

ppm. Rates of net photosynthesis, stomatal conductance and transpiration were recorded at photon flux densities ranging from 1600 to 30 μmol m^{-2} s^{-1}. Immediately after measurements were taken, leaves were detached and their individual areas determined using a Delta T area meter (Delta-T Devices Ltd., Cambridge, U.K.). A light response curve for the *Eucalyptus* was constructed to determine the light saturation point, Q_l.

With further measurements made in the pot trial, a non-rectifiable problem with the flow controllers of the PAC flow control system was found to yield unrealistic internal CO_2 concentrations. This finding suggests a certain amount of doubt in the accuracy of the values recorded for net photosynthesis at the different light intensities, since $p_i = p_a - A/g$, where p_i is the internal CO_2 concentration in the leaf mesophyll, p_a is the ambient CO_2 concentration passing over the leaf, A is the net photosynthetic rate and g is the conductance to CO_2. However, it is justifiable to assume that the light saturation point, (Q_l), derived from this data set would not be affected, since although the net photosynthetic rates measured may be open to internal error, the scale upon which the values were derived was uniform throughout the determination of Q_l.

Subsequently accurate rates of net photosynthesis, stomatal conductance and transpiration were measured above saturating light intensities (A_{max}) using an LCA2 infra red gas analyser (IRGA), air supply unit (ASU), and a narrow Parkinson leaf chamber (The Analytic Development Co. Ltd. Hoddeson, England). Leaf chamber conditions followed ambient air temperatures ranging between 19 and 34^{0}C and CO_2 concentrations of approximately 350 ppm. Due to the design of the ADC system, moisture was removed from the air passing into the leaf chamber with the use of anhydrous calcium sulphate traps which meant that the leaf was exposed to dry air for one minute whilst measurements were made. Ambient atmospheric relative humidities measured with an Asman psychrometer at the study site at hourly intervals over the period that photosynthetic measurements were made were low (27% $\pm$ 10.2, n=28) due to the effects of the 1991/1992 summer drought. Gas exchange measurements were taken once on the same leaf category and canopy position as chosen for the construction of the light response curve on three trees of each clone and treatment between 6th and 12th July 1992.

2.2.10 *Leaf xylem water potentials*

Leaf xylem water potentials were measured using a pressure chamber (PMS Instrument Co. model 1000) on three healthy leaves per plant on the morning (4:00 to 6:00 am) that leaf gaseous exchange measurements were to be made. Immediately after leaf gaseous exchange measurements were made the leaf xylem water potentials were measured on three more healthy leaves in the canopy.

2.2.11 *Foliar nitrogen*

Foliar nitrogen analyses were performed on 100 mg of a dried subsample of leaves chosen to determine specific leaf areas, using the Kjeldahl method (Smith, 1980) in the Botany Department of the University of Cape Town. Canopy foliar nitrogen content was calculated as the product of leaf nitrogen concentration per unit leaf mass and canopy leaf dry weight.

2.2.11 **Statistical analyses**

Statistical analyses, data manipulation and graphic display were performed using the STATGRAPHICS (Version 5.0, Statistical Graphics Corp., Maryland, USA) and QUATRO PRO (Version 4.0, Borland International INC., California, USA) software packages.

2.2.11.1 *Light response curve*

A Non-linear Regression was used to plot the hyperbolic curve representing the relationship between assimilation rate per unit area and photosynthetic active radiation using the hyperbolic function of Jarvis (1976).

2.2.11.2 *Parameters describing treatment and clonal effects*

Two Way and One Way Analyses of Variances were performed to determine the combined or individual effects of water availability or genotype on growth variables after which a Tukey Multiple Range test was performed on means to detect significant differences (Zar, 1984).

2.2.11.2 *Relationship among variables*

Multiple correlations were used to determine significant correlations amongst the growth variables after which Simple Linear Regression analyses were carried out to establish the

relative dependence of the variable in question on another. Stepwise Linear Multiple Regression models were used to determine the combined effects of significant variables on RGR and wood dry mass production in each treatment (Zar, 1984).

2.3 RESULTS

2.3.1 Water use, growth and dry mass allocation

Water availability had a significant effect on the mean total water uptake ($F_{1,34} = 155.4$, $p < 0.05$) which had a significant influence on mean total dry mass accumulated ($F_{1,34} = 90.3$, $p < 0.05$), mean relative growth rate ($F_{1,34} = 90.2$, $p < 0.05$) and partitioning to leaves ($F_{1,34} = 16.6$, $p < 0.05$), branches ($F_{1,34} = 6.6$, $p < 0.05$), main stems ($F_{1,34} = 28.1$, $p < 0.05$) and roots ($F_{1,34} = 52.3$, $p < 0.05$) amongst high (W_h) and low (W_l) treatment plants (Table 2.2). Root:shoot and root:leaf area ratios were significantly higher in W_h than W_l ($F_{1,34} = 7.3$, $p < 0.05$ and $F_{1,34} = 4.6$, $p < 0.05$). The availability of water did not have a significant effect on mean LAR ($F_{1,34} = 2.3$, $p > 0.1$) whereas mean NAR was slightly higher in individuals of W_h than W_l ($F_{1,34} = 3.0$, $p < 0.05$) (Table 2.2).

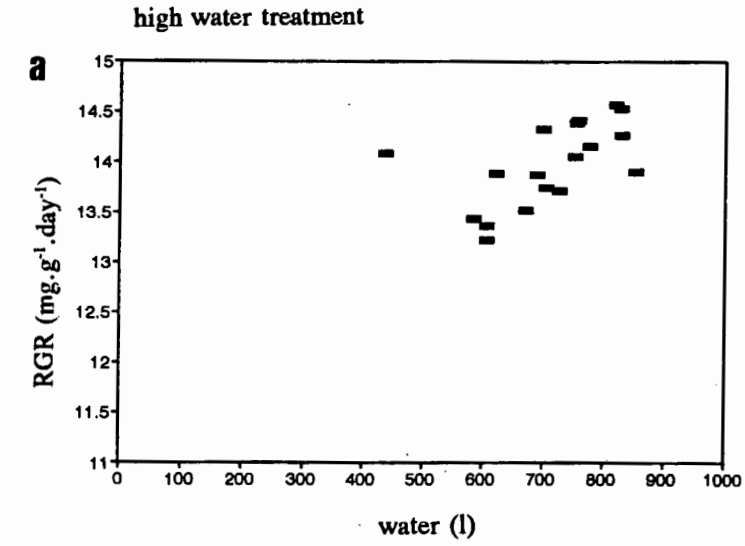
A mean reduction in water usage of 55.2% in W_l relative to W_h plants resulted in a mean reduction of 10.1% in RGR and 48.2% in total dry mass. The mean reduction in water usage closely paralleled the 54.5% reduction in root dry mass which was followed by a 45.4% mean reduction in main stem dry mass, 42.2% reduction in leaf dry mass and 23.6% reduction in branch dry mass. Mean root:shoot and root:leaf area ratios of the individuals of W_l were 26.6% and 26.4% lower than the individuals of W_h respectively.

RGR correlated significantly and positively with water uptake in both the high and low water treatments ($p < 0.05$) (Figure 2.2 a and b). Significant positive correlations were found between RGR and root and shoot dry mass in both W_h and W_l (Figures 2.3 a - d), however allocation to root dry mass was a stronger determinant of RGR than shoot dry mass in W_l. Despite the significant relationships found between RGR and allocation to below and above ground dry mass components, RGR did not correlate significantly with the allocation to root:shoot and root:leaf area ratios in W_h ($p > 0.05$). Similarly no significant relationship

was found between RGR and root:shoot ratios in W_l ($p > 0.05$) but RGR was significantly correlated with root:leaf area ratio ($p < 0.05$) (Figures 2.4 a - d).

Table 2.2 RGR, biomass accumulation and partitioning to above- and below ground plant components in high and low water treatments. Mean values with standard errors for each treatment are given. A single factor analysis of variance was performed on each variable between treatments. Different letters indicate means are significantly different at $p < 0.05$ (Tukey Multiple Range test).

Variable	Treatment	
	High	Low
Water use (litres over 16 mth)	$706^a \pm 27.65$	$316^b \pm 13.37$
RGR ($mg.g.day^{-1}$)	$14.23^a \pm 0.11$	$12.81^b \pm 0.09$
Total biomass (g)	$2026.16^a \pm 87.59$	$1051.33^b \pm 53.39$
Leaves (g)	$276.28^a \pm 24.80$	$159.74^b \pm 14.26$
Branches (g)	$200.44^a \pm 16.36$	$153.06^b \pm 8.61$
Main stem (g)	$373.45^a \pm 28.35$	$203.84^b \pm 14.82$
Roots (g)	$1175.99^a \pm 78.53$	$534.69^b \pm 41.15$
Root:shoot ($g.g^{-1}$)	$1.43^a \pm 0.08$	$1.05^b \pm 0.04$
LWR ($g.g^{-1}$)	$0.14^a \pm 0.01$	$0.16^a \pm 0.02$
Root:leaf area ($g.m^{-2}$)	$693.80^a \pm 69.27$	$510.63^b \pm 50.53$
LAR ($m^2.kg^{-1}$)	$0.84^a \pm 0.64$	$0.10^a \pm 0.99$
NAR ($g.\ m^{-2}.day^{-1}$)	$2.41^a \pm 0.17$	$2.02^a \pm 0.15$



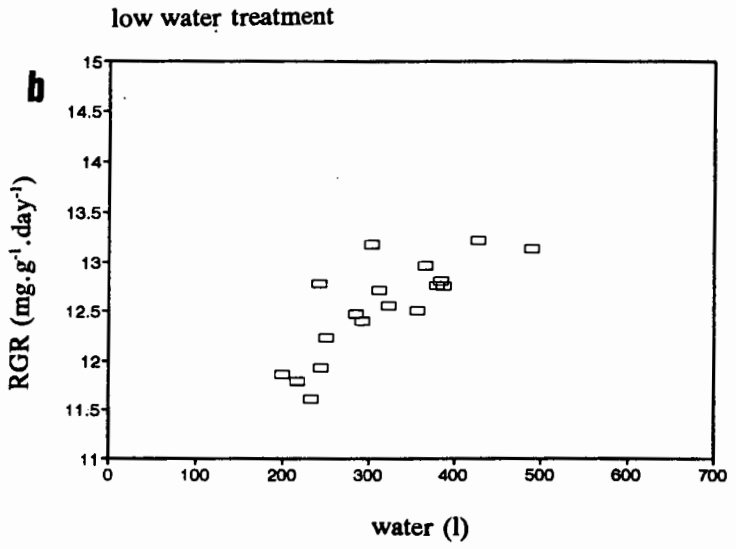


Figure 2.2 Relationship between RGR and water uptake for individuals of W_h (a) RGR = 0.002 water + 12.46, r = 0.55, $p < 0.05$; and W_l (b), RGR = 0.005 water + 11.01, r = 0.79, $p < 0.05$.

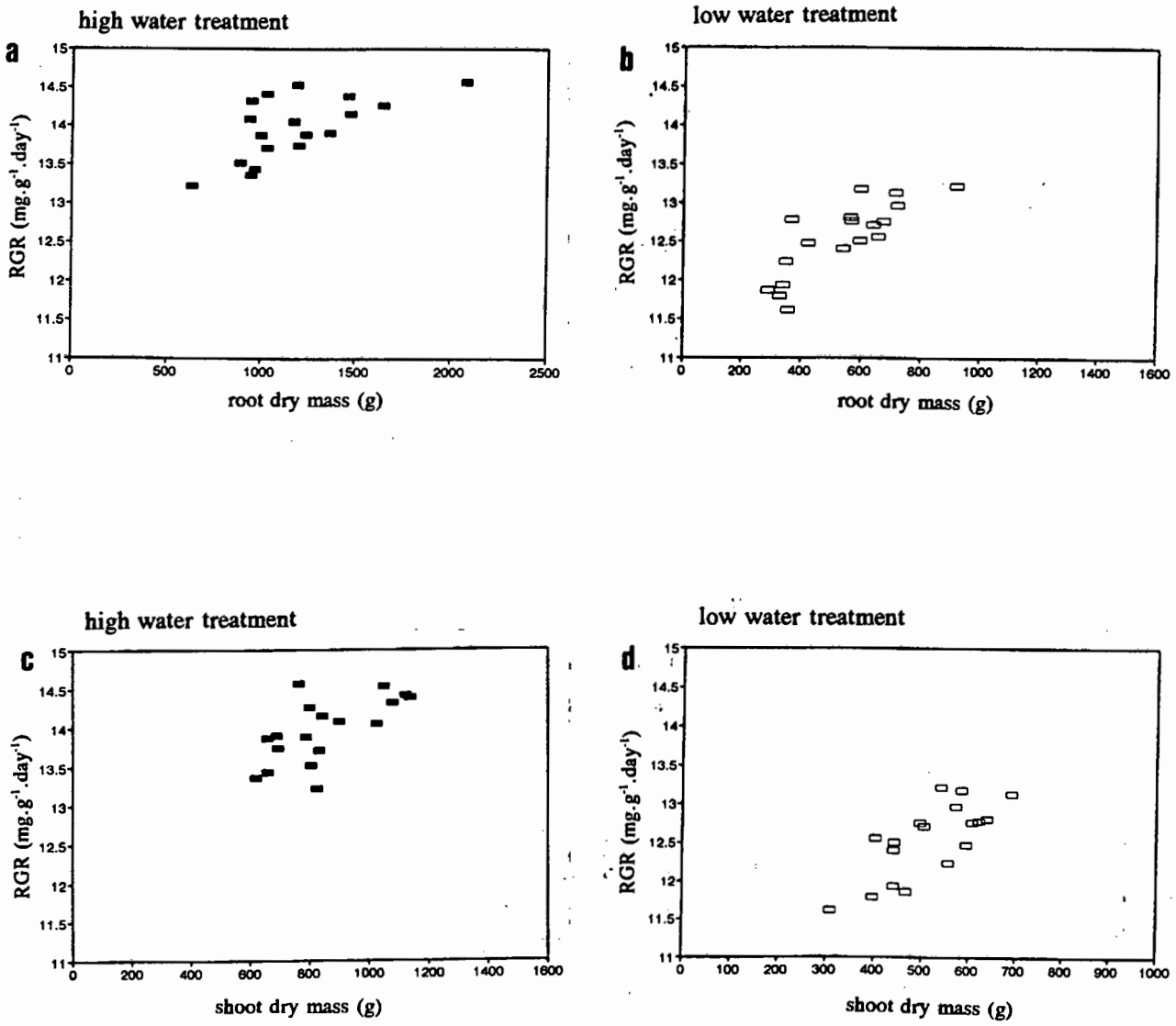


Figure 2.3 Relationship between RGR and root biomass for individuals of W_h (a), RGR = 0.0008 root + 13.02, r = 0.64, $p < 0.05$ and W_l (b), RGR = 0.002 root + 11.32, r = 0.81, $p < 0.05$. Relationship between RGR and shoot biomass for individuals of W_h (c), RGR = 0.002 shoot + 12.65, r = 0.63, $p < 0.05$ and W_l (d), RGR = 0.004 shoot + 10.66, r = 0.74, $p < 0.05$.

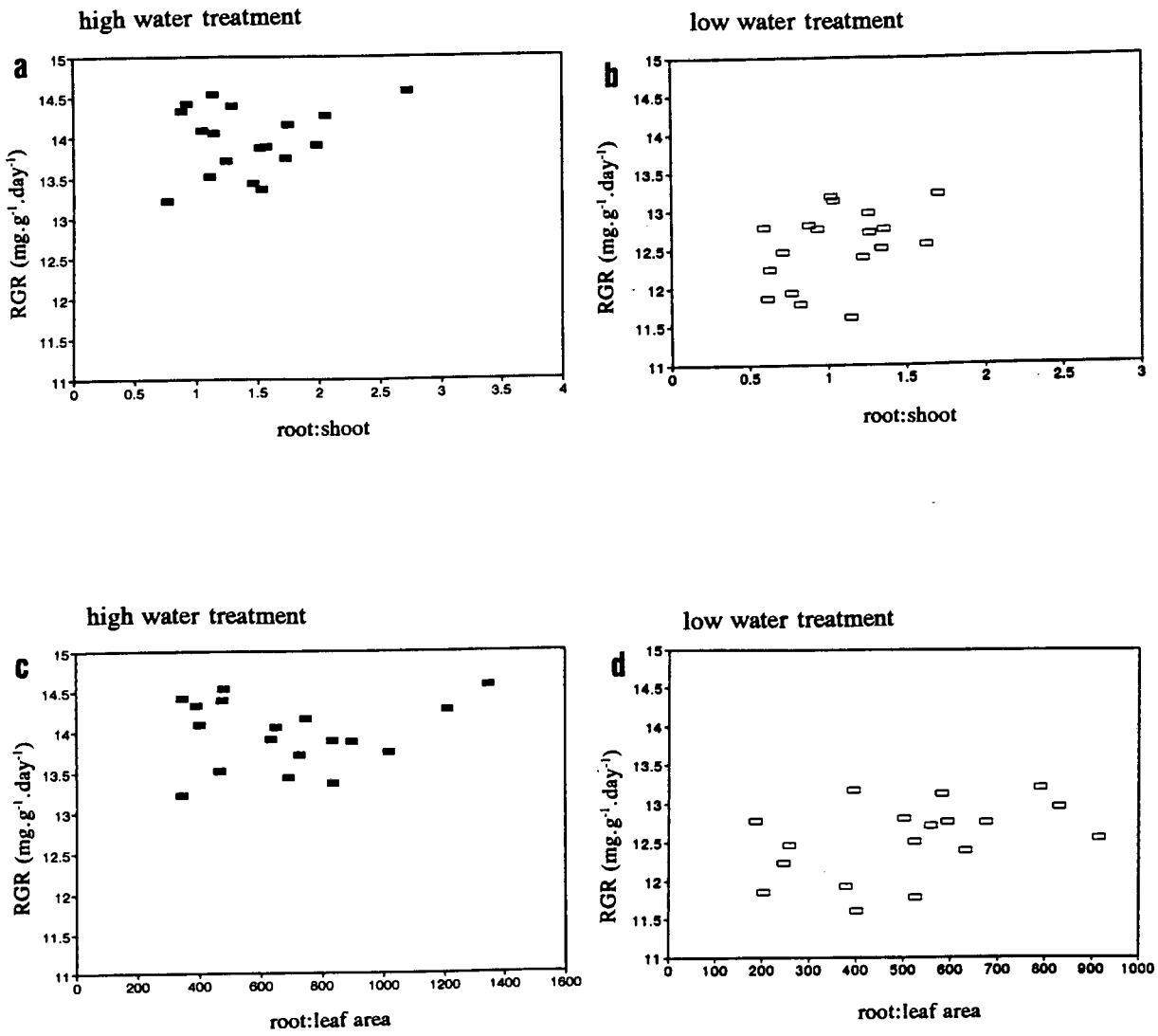


Figure 2.4 Relationship between RGR and root:shoot ratios for individuals of W_h (a), r = 0.22, $p > 0.05$; and W_l (b), r = 0.39, $p > 0.05$. Relationship between RGR and root:leaf area ratio for individuals of W_h (c), r = 0.103, $p > 0.05$ and W_l (d), RGR = 0.0009 root:leaf area ratio, r = 0.41, $p < 0.05$.

2.3.2 Canopy morphology and structure

Carbon allocation to photosynthetic and non-photosynthetic tissue in the plant (LWR) was not significantly affected by water availability ($F_{1,34} = 0.8$, $p > 0.05$) under both treatments (Table 2.2). Similarly water availability did not have a significant effect on the leaf area index ($F_{1,34} = 0.002$, $p > 0.05$) (Table 2.3). A significant effect of water availability was found in the canopy leaf area amongst the two treatments ($F_{1,34} = 19.4$, $p < 0.05$) where the reduction in water availability resulted in a 39% decrease in canopy leaf area (Table 3). However this may be a direct result of the overall reduction in dry mass, including leaf dry mass with lower water availability. Specific leaf areas, were not significantly affected by water availability ($F_{1,34} = 1.1$, $p > 0.05$). SLA and LWR were significantly and negatively correlated with each other in W_h ($p < 0.05$) but not in W_l ($p > 0.05$, not shown). NAR and LAR were correlated negatively under both W_h and W_l ($p < 0.05$, not shown).

Significant relationships between RGR and significant variables relating to canopy morphology and structure are shown in Figures 2.5 a - d. No significant relationships were found between RGR and LWR or leaf area index in both W_h and W_l ($p > 0.05$, not shown). An increase in RGR was significantly correlated with an increase in canopy leaf area in W_h ($p < 0.05$), whereas this relationship was not significant in W_l ($p > 0.05$) (Figures 2.5 a and b). In W_l, RGR increased significantly with a decrease in SLA ($p < 0.05$), whereas this relationship was not found in W_h ($p > 0.05$) (Figures 2.5 c and d).

2.3.3. Gaseous exchange measurements, leaf nitrogen and plant water status

The net photosynthetic light response curve for *E. grandis* measured in this study is shown in Figure 2.6. Light saturation (Q_l) was estimated at approximately 1200 $\mu mol.m^{-2}.sec^{-1}$.

Mean net leaf photosynthetic rates (A_{max}) recorded were significantly higher by 40% in W_h than W_l ($F1,34 = 4.5$, $p < 0.05$) which coincided with a 54% increase in stomatal conductance ($F_{1,34} = 8.0$, $p < 0.05$), 59% increase in rates of transpiration ($F_{1,34} = 14.9$, $p < 0.05$) and a 45% increase in $XPP_{difference}$ ($F_{1,34} = 6.6$, $p < 0.05$) (Table 2.3).

Table 2.3 Parameters of canopy structure, leaf morphology and physiology measured on individuals of high and low water treatments. Mean values with standard errors are given. A single factor analysis of variance was performed on each variable between treatments. Different letters indicate means are significantly different at $p < 0.05$ (Tukey Multiple Range test).

Variable	Treatment	
	High water	Low water
Leaf area index (LAI)	$1.01^a \pm 0.11$	$0.99^a \pm 0.16$
Canopy leaf area (m^2)	$1.87^a \pm 0.14$	$1.14^b \pm 0.08$
Specific leaf area ($m^2.kg^{-1}$)	$6.24^a \pm 1.97$	$6.58^a \pm 1.64$
Leaf nitrogen concentration ($mg.g^{-1}$ dry wt)	$17.83^a \pm 0.61$	$28.58^b \pm 0.99$
Canopy nitrogen content (g)	$4.98^a \pm 0.51$	$4.46^a \pm 0.37$
Amax ($\mu mol.m^{-2}.sec^{-1}$)	$5.04^a \pm 0.59$	$3.04^b \pm 0.71$
Gs ($mmol.m^{-2}.sec^{-1}$)	$109.34^a \pm 15.2$	$50.28^b \pm 14.35$
E ($mmol.m^{-2}.\ sec^{-1}$)	$19.66^a \pm 2.19$	$8.12^b \pm 1.77$
Predawn leaf xylem water potential (XPP) (bars)	$-5.65^a \pm 1.12$	$-12.55^b \pm 4.82$
Midday leaf xylem water potential (XPP) (bars)	$-20.76^a \pm 3.97$	-21.91a $\pm$ 4.20
Leaf $XPP_{difference}$* (bars)	$15.11^a \pm 1.63$	$8.27^b \pm 2.10$

Leaf $XPP_{difference}$* is the shift in leaf xylem water potential from predawn to midday

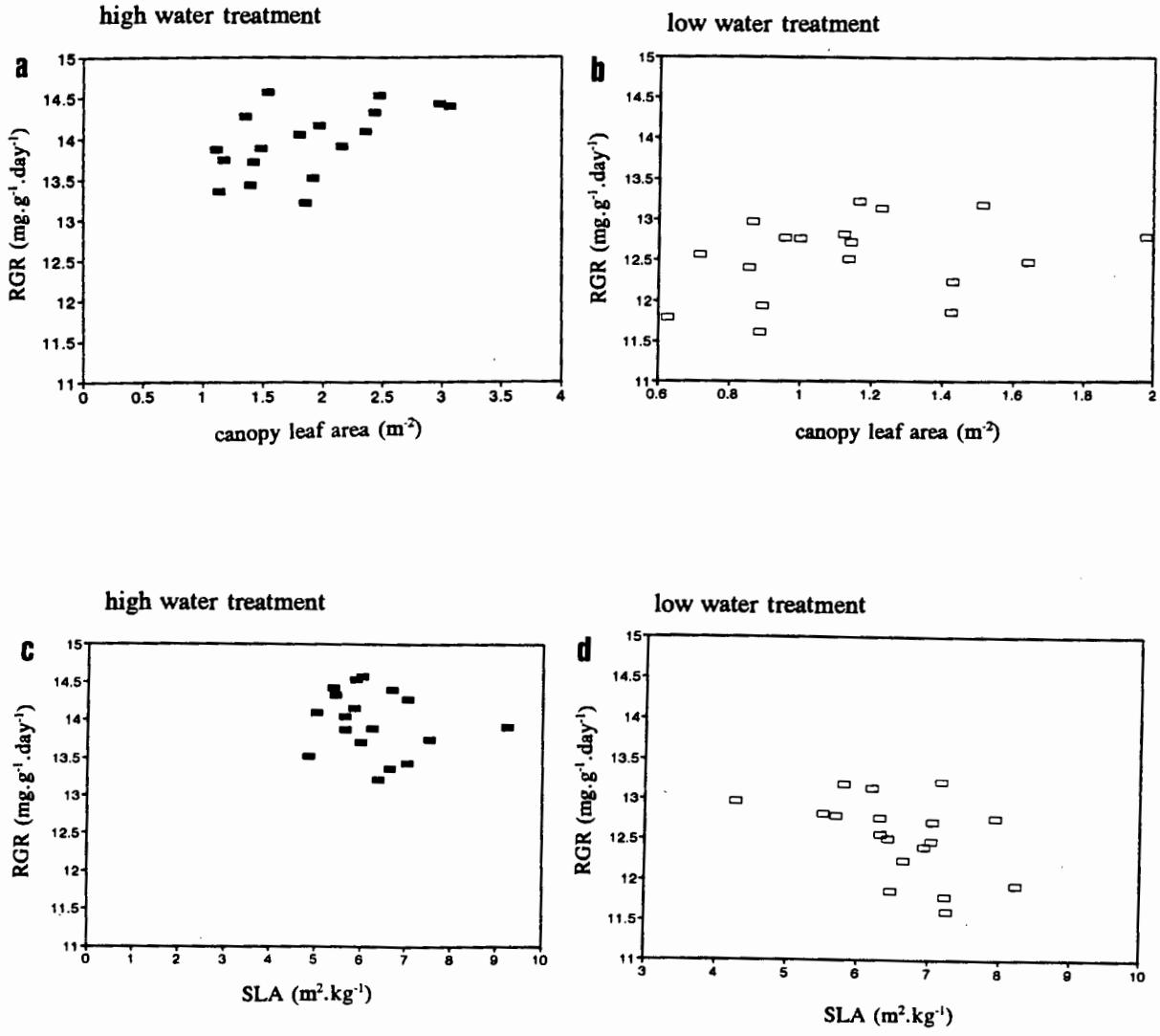


Figure 2.5 Relationship between RGR and canopy leaf area for individuals of W_h (a), RGR = 0.37 canopy leaf area + 13.27, $r = 0.54$, $p < 0.05$; and for individuals of W_l (b), $r = 0.28$, $p > 0.05$. Relationship between RGR and SLA for individuals of W_h (c), $r = -0.19$, $p > 0.05$, and W_l (d), RGR = 14.19 - 0.025 SLA, $r = -0.48$, $p < 0.05$.

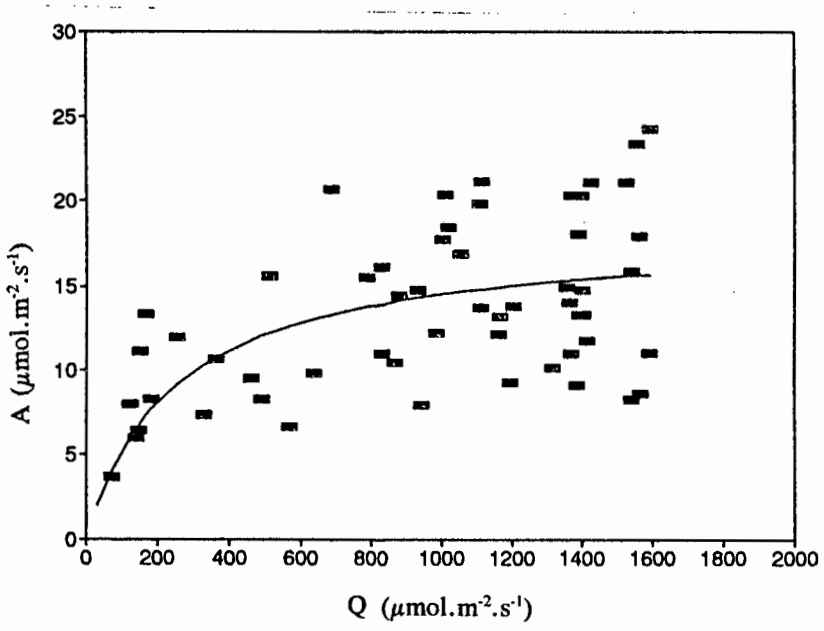


Figure 2.6 Light response curve for clones of *Eucalyptus* in pot trial. $A = ß_1ß_2\ Q\ /\ ß_1 + (ß_2\ Q)$ (Jarvis, 1976).

On average, leaf nitrogen concentrations were significantly higher by 40% in individuals of W_l than in W_h ($F_{1,34} = 85.5$, $p < 0.05$). However mean leaf canopy nitrogen contents were similar ($F_{1,34} = 0.7$, $p > 0.1$) between W_h and W_l (Table 2.3), suggesting that the concentration differences resulted mainly from a dilution or concentration effect with higher or lower leaf dry mass in the respective treatments.

RGR did not correlate significantly with A_{max} in both W_h and W_l (Figures 2.7 a - b), whereas RGR was found to be significantly and negatively correlated with leaf nitrogen concentration in W_l ($p < 0.05$) (Figure 2.7 d). This relationship was not significant in W_h ($p > 0.05$) (Figure 2.7 c).

A_{max} and nitrogen per unit leaf mass and area did not correlate significantly in both W_h and W_l ($p > 0.05$) (Figures 2.8 a - d). A_{max} correlated negatively with SLA in W_l ($p < 0.05$) which may be attributed to higher nitrogen per unit area at lower SLA (Figures 2.9 c and d). The relationship between A_{max} and SLA was not significant in W_h ($p > 0.05$) despite the significant correlation between SLA and leaf nitrogen expressed per unit area ($p < 0.05$) (Figures 2.9 a and b). Instead it was found that A_{max} was strongly correlated ($p < 0.05$) with rates of stomatal conductance and transpiration (Figures 2.10 a - d). This implies that

A_{max} was strongly influenced by the stomatal control on rates of stomatal conductance and transpiration which was functionally dependent on plant water status (Figures 2.10 e - f) rather than on foliar nitrogen concentrations.

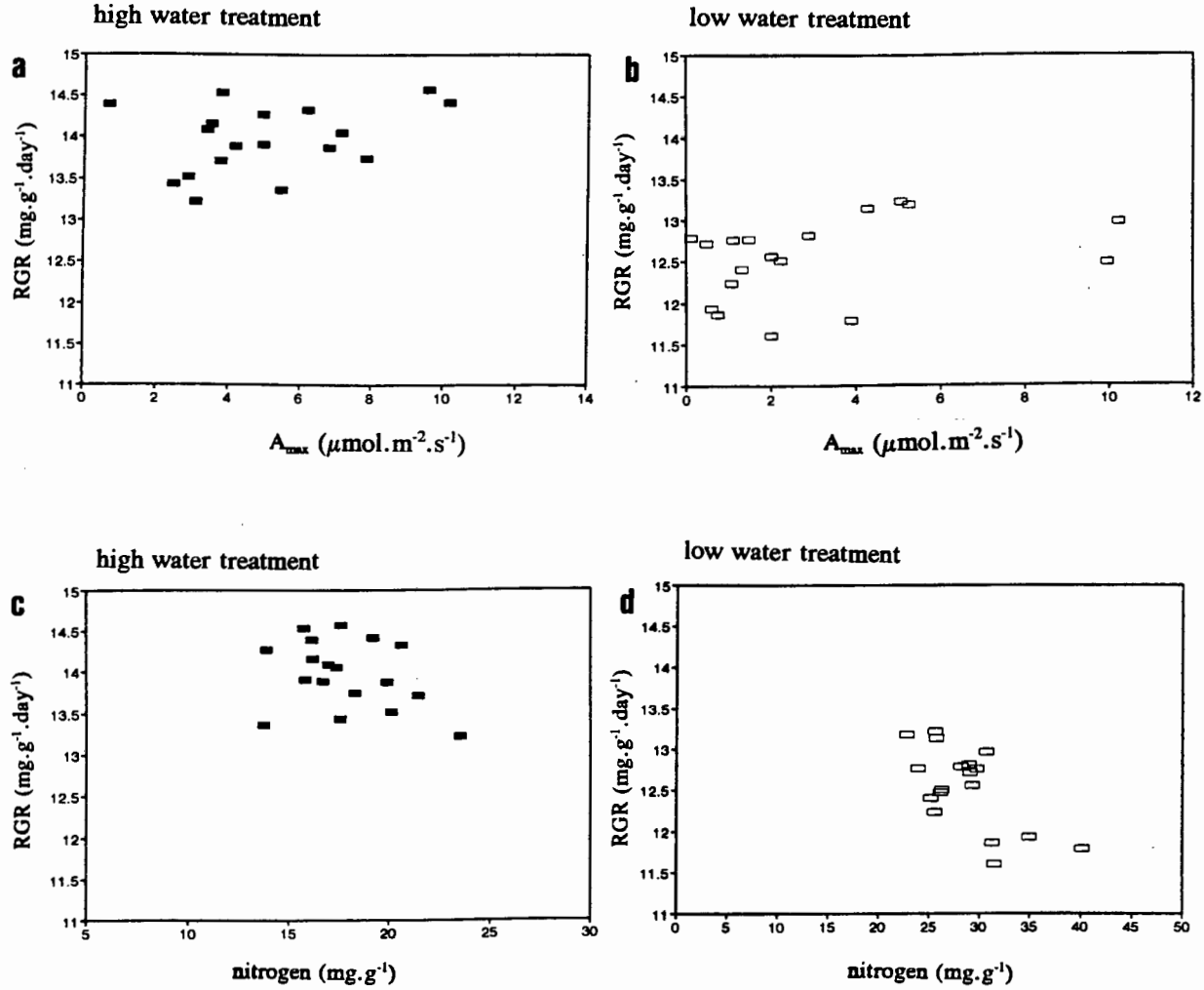


Figure 2.7 Relationship between RGR and A_{max} for individuals of W_h (a), $r = 0.32$, $p > 0.05$; and for individuals of W_l (b), $r = 0.03$, $p > 0.05$. Relationship between RGR and leaf nitrogen concentrations for individuals of W_h (c), $r = -0.32$; $p > 0.05$; and for individuals of W_l (d), RGR = 14.69 - 0.076 nitrogen, $r = -0.65$, $p < 0.05$.

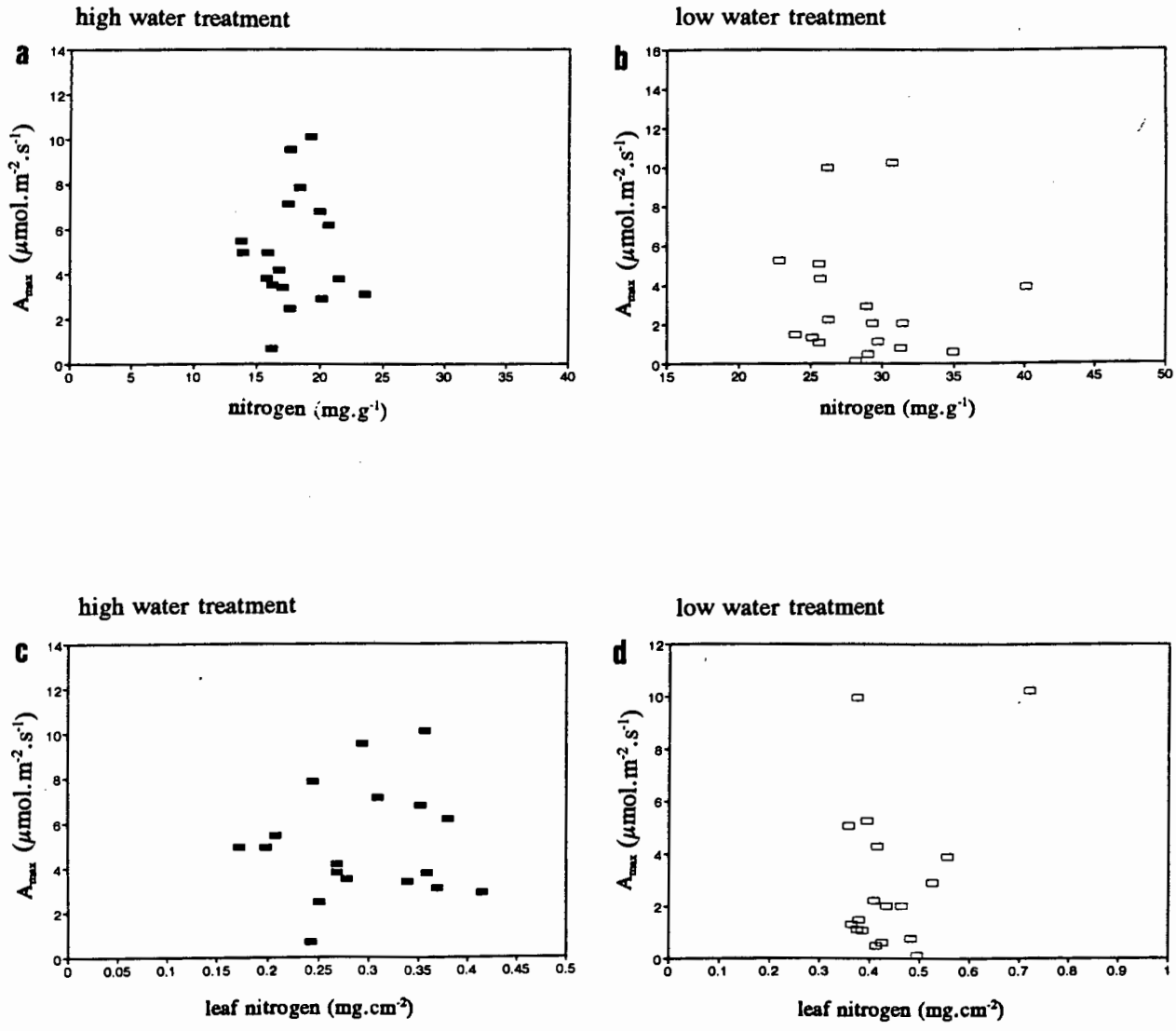


Figure 2.8 Relationship between A_{max} and nitrogen per unit leaf mass for individuals of W_h (a), $r = 0.06$, $p > 0.05$; and for individuals of W_l (b), $r = -0.09$, $p > 0.05$. Relationship between A_{max} and nitrogen per unit leaf area for individuals of W_h (c), $r = 0.08$, $p > 0.05$, and for individuals of W_l (d), $r = 0.36$, $p > 0.05$.

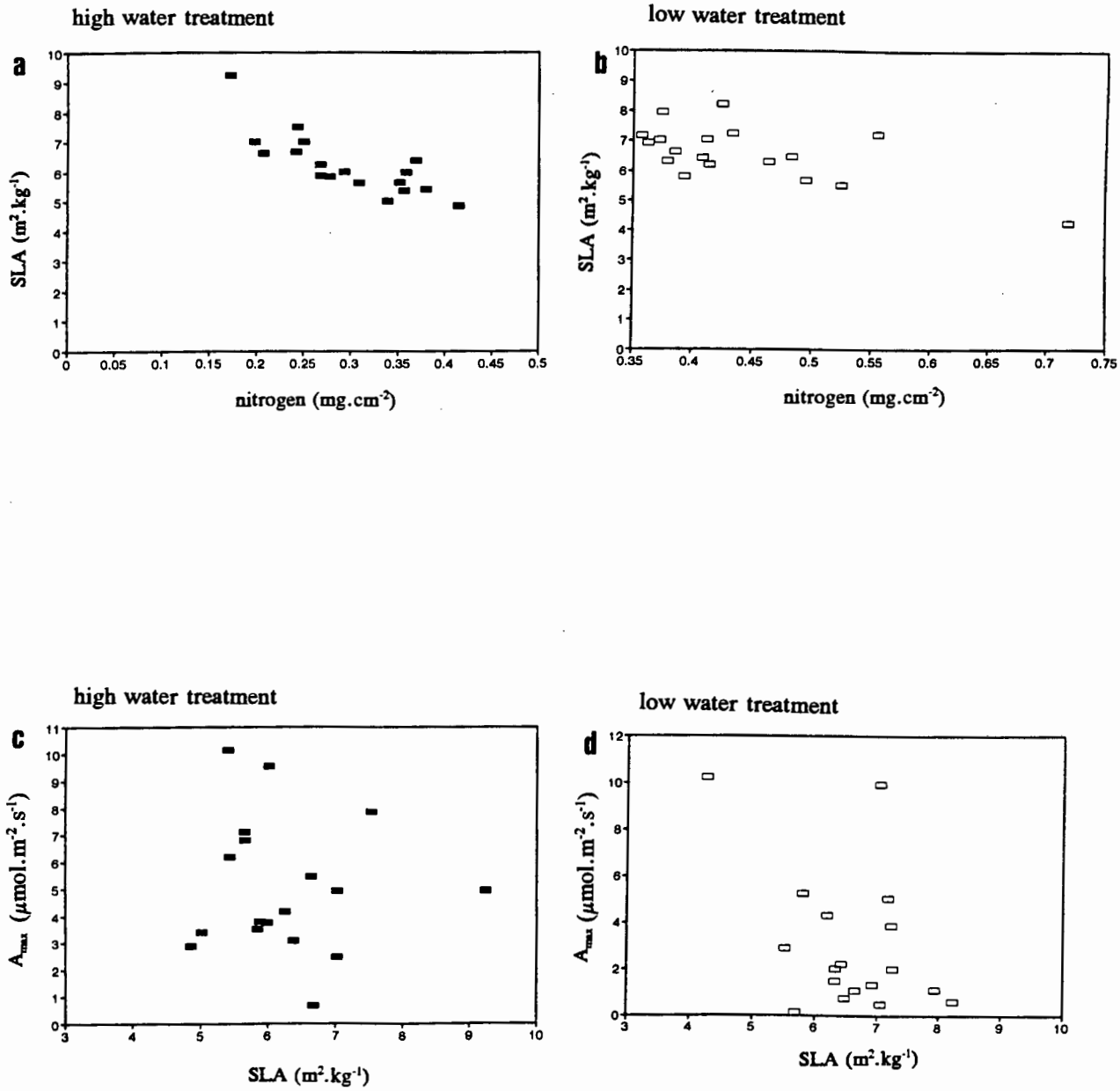


Figure 2.9 Relationship between SLA and nitrogen per unit leaf area for individuals of W_h (a), SLA = 97.59 nitrogen - 119.41, r = - 0.80, $p < 0.05$; and for individuals of W_l (b), SLA = 94.63 - 65.24 nitrogen, r = - 0.63, $p < 0.05$. Relationship between A_{max} and SLA for individuals of W_h (c), r = - 0.06, $p > 0.05$, and for individuals of W_l (d), A_{max} = 11.88 - 0.13 SLA, r = 0.41, $p < 0.05$.

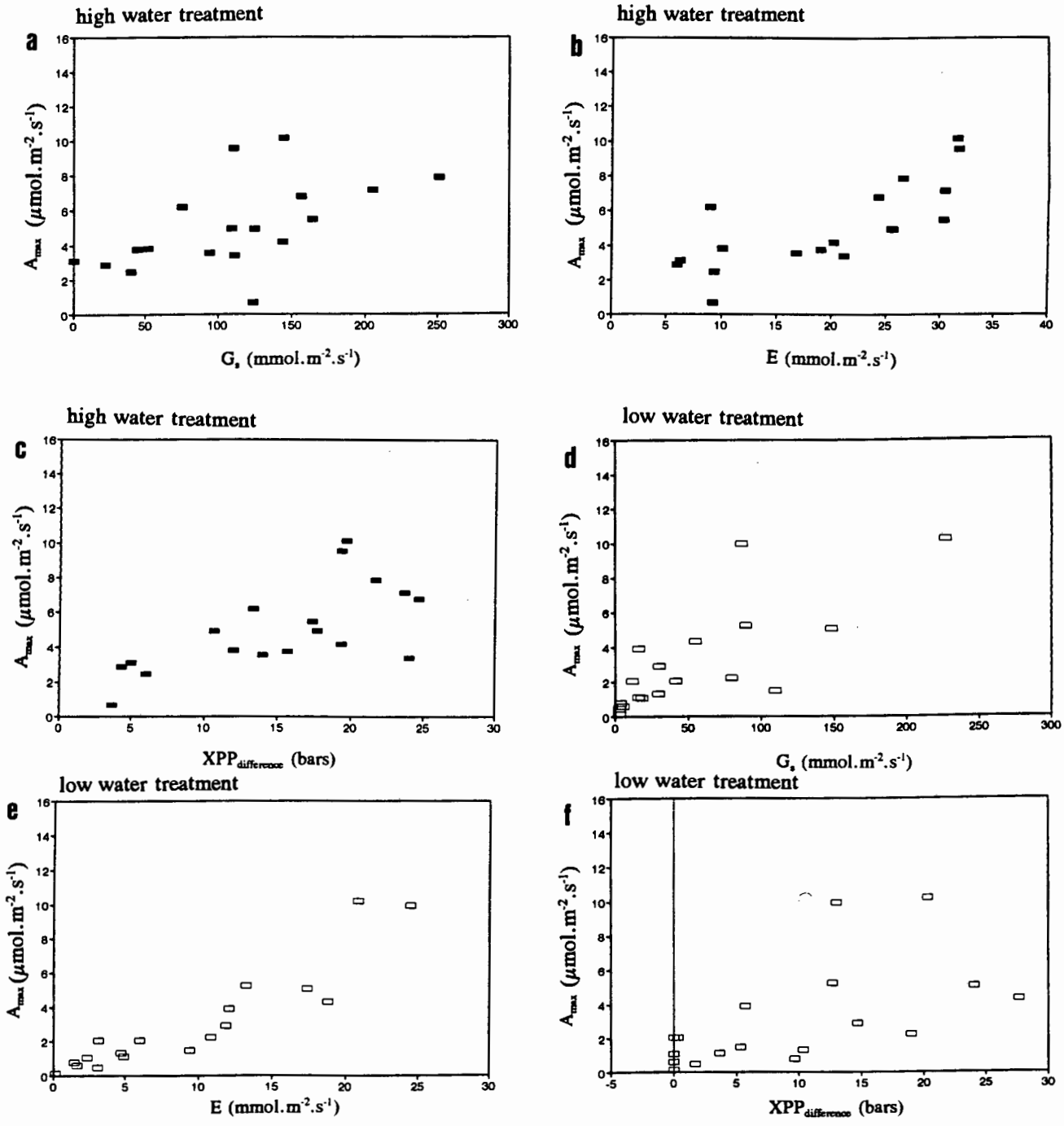


Figure 2.10. Relationships between A_{max} and G_s (a) A_{max} = 0.021 Gs + 2.71, r = 0.55, p < 0.05; A_{max} and E (b), A_{max} = 0.204 E + 1.03, r = 0.757, p < 0.05, A_{max} and $XPP_{difference}$ (c), A_{max} = 0.241 $XPP_{difference}$ + 1.4, r = 0.669, p < 0.05, for individuals of W_h. Relationships between A_{max} and G_s (d), A_{max} = 0.037 Gs + 0.99, r = 0.764, p < 0.05, A_{max} and E (e), A_{max} = 0.37 E - 0.37, r = 0.91, p < 0.05, A_{max} and $XPP_{difference}$ (f), A_{max} = 0.2 $XPP_{difference}$ + 1.17, r = 0.60, p < 0.05, for individuals of W_l.

2.3.4 Growth traits contributing to RGR

Significant Multiple Regressions for the relationship between growth traits described in the previous sections, and RGR are shown in Table 2.4. The use of a Stepwise Multiple Linear Regression model showed that under relatively high water availability, RGR is mediated through changes in canopy leaf area and NAR ($r^2 = 0.84$). With the exclusion of NAR from the model, canopy leaf area explained only 25% of the variance in RGR. Significant components of NAR such as increases in root:leaf area ratio, decreases in SLA together with increases in canopy leaf area explained 81% of the variation in RGR. However the contribution of SLA to RGR is small (5%) in comparison to canopy leaf area and root:leaf area ratios (76%). Under lower moisture availability (W_l), RGR is determined by changes in foliar nitrogen concentrations, NAR, canopy leaf area and LAR ($r^2 = 0.92$) (Table 2.4). The effects of the components of NAR and LAR i.e. root:leaf area ratios, LWR and SLA, are pronounced with the removal of NAR and LAR from the model (Table 2.4). The cumulative effects of increases in root:leaf area ratios and canopy leaf area with simultaneous decreases in LWR and SLA explained 88% of the variation in RGR. However the effects of LWR and SLA contributed a small percentage (11%) to the explained variance of RGR relative to the additive effects of root:leaf area ratios and canopy leaf area (77%).

The results from the model estimations indicate that increases in RGR of eucalypt seedlings are achieved mainly through large canopy leaf areas, large root:leaf area ratios, lower SLA (i.e. the production of thick leaves) and higher NAR under high and low moisture availability.

2.3.5 Clonal variation in the relative allocation of dry mass across treatments

The absolute values for mean RGR and dry mass production for whole plant and the different plant organs after 16 months are shown in Table 2.5. No significant clonal variation was found in RGR and total dry mass. Clonal variation in dry mass allocation to main stem (harvestable dry mass), branches, leaves and roots were significantly different suggesting that there may be scope for manipulating allocation patterns to attain greater yields of harvestable dry mass.

Table 2.4 Stepwise Multiple Regression Model estimation of the significant growth characteristics influencing RGR at $p < 0.05$, of *Eucalyptus* seedlings under high and low water availability. Regression equations and coefficients of determination (r^2) for the multiple effects of the significant variables are given.

Regression	r^2
High water treatment (W_h)	
RGR = 0.373 canopy leaf area + 13.27	0.25
RGR = 1.103 canopy leaf area + 0.684 NAR + 10.429	0.84
Subcomponents of NAR	
RGR = 0.851 canopy leaf area + 0.002 root:leaf area + 11.417	0.76
RGR = 0.854 canopy leaf area + 0.002 root:leaf area - 0.010 SLA + 11.967	0.81
Low water treatment (W_l)	
RGR = 14.699 - 0.076 nitrogen	0.38
RGR = 14.120 - 0.079 nitrogen + 0.340 NAR	0.55
RGR = 9.82 - 0.029 nitrogen + 0.928 NAR + 1.468 canopy leaf area	0.88
RGR = 10.611 - 0.023 nitrogen + 0.645 NAR - 0.057 LAR	0.92
Subcomponents of NAR and LAR	
RGR = 0.0009 root:leaf area + 12.05	0.12
RGR = 0.0027 root:leaf area + 1.539 canopy leaf area + 9.416	0.77
RGR = 0.0021 root:leaf area + 1.933 canopy leaf area - 4.284 LWR + 9.969	0.83
RGR = 0.002 root:leaf area + 1.865 canopy leaf area - 6.139 LWR - 0.015 SLA + 11.632	0.88

Table 2.5 Mean relative growth rates and biomass accumulation of *Eucalyptus* clones after 16 months under high and low watering treatments. Values are means with SE in parenthesis and the different letters indicate significant differences for each growth variable among clones (a,b,c) (Tukey multiple range with significance at $\alpha = 0.05$ following One-way ANOVA) or between treatments (x,y) (Tukey multiple range with significance at $\alpha = 0.05$ following the Two-way ANOVA). 1, clonal means are not significantly different in both high and low water treatments ($F_{5,12} = 0.457$ and 2.914, respectively, $p > 0.05$). 2, clonal means are not significantly different in both high and low watering treatments ($F_{5,12} = 0.418$ and 2.921 respectively, $p < 0.05$). 3, clonal means are significantly different in high and low watering treatments ($F_{5,12} = 9.246$ and 4.222, respectively, $p < 0.05$). 4, clonal means are significantly different in high water treatment ($F_{5,12} = 11.09$, $p < 0.05$) but no significant differences are found in the low water treatment ($F_{5,12} = 1.073$, $p > 0.05$). 5, Clonal means are not significantly different in the high watering treatment ($F_{5,12} = 1.688$, $P > 0.05$) but significant differences are found in the low water treatment ($F = 5.604$, $p < 0.05$).

Clone	RGR[1] (mg g^{-1} day^{-1})		Whole plant[2] (g)		Main stem[3] (g)		Side stem[4] (g)		Leaf[5] (g)		Root[6] (g)	
	High[x]	Low[y]	High[x]	Low[y]	High[x]	Low[y]	High[x]	Low[y]	High[x]	Low[y]	High[x]	Low[y]
1	14.15a (0.10)	13.37a (0.07)	1905a (157)	1258a (59)	527b (74)	319b (15)	86b (16)	137a (22)	226a (30)	177ab (8)	1066a (52)	665ab (51)
2	14.27a (0.20)	12.42a (0.31)	2171a (216)	893a (141)	390ab (31)	198a (26)	264a (36)	183a (16)	220a (69)	101a (21)	1297a (81)	412a (78)
3	14.20a (0.29)	13.11a (0.19)	2108a (273)	1229a (119)	390ab (51)	203a (12)	173ab (10)	152a (18)	192a (24)	124a (17)	1353a (209)	750b (85)
4	14.24a (0.33)	12.81a (0.09)	2171a (355)	1053a (48)	223a (19)	167a (7)	240a (26)	176a (14)	240a (9)	119a (22)	1467a (325)	590ab (29)
5	14.03a (0.40)	12.37a (0.26)	1797a (230)	862a (103)	305ab (46)	150a (29)	253a (9)	137a (34)	337ab (35)	199ab (34)	902a (160)	375a (23)
6	14.55a (0.10)	12.74a (0.31)	2006a (91)	973a (123)	405ab (58)	186a (18)	187a (9)	132a (12)	443b (33)	239b (26)	971a (28)	416a (92)

2.3.6 Clonal variation in the relative allocation of dry mass across treatments

The expression of organ dry mass as a percentage allocation of whole plant dry mass provides a comparative analysis of clonal allocation patterns under the different water treatments (Figure 2.11). The average relative dry mass allocation across clone and treatment approximated 54% to roots, 19% to main stem (H), 13% to branch and 15% to leaves. Significant differences among clones were found in the allocation to root dry mass in both the high and low water treatments ($F_{5,12}$ = 7.8 and 6.4 respectively, $p < 0.05$); to leaf dry mass in both high and low water treatments ($F_{5,12}$ = 13.0 and 17.7 respectively, $p < 0.05$); to harvestable stem dry mass in high and low water treatments ($F_{5,12}$ = 12.9 and 6.7 respectively, $p < 0.05$) and to branch dry mass in both high and low water treatments ($F_{5,12}$ = 9.9 and 5.0 respectively, $p < 0.05$).

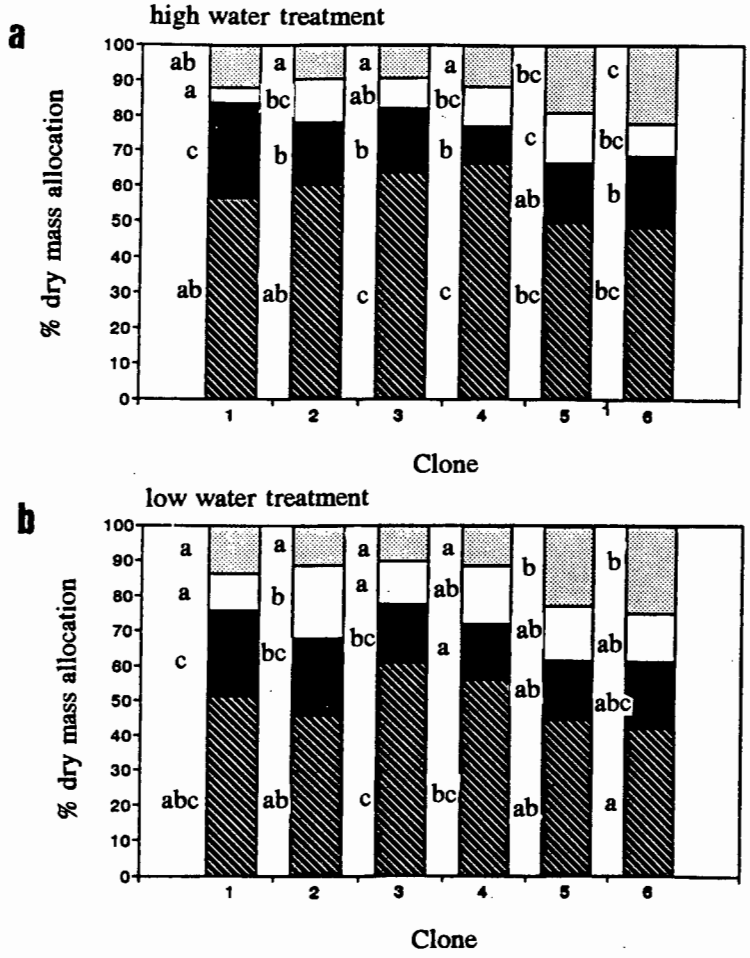


Figure 2.11 Relative mean clonal allocation to roots ▨ , main stems ■ , branches □ , and leaves ▩ for individuals of W_h (a) and W_l (b). Different letters indicate significant differences among clones at each organ level (Tukey Multiple Range test following one way ANOVA of arcsine transformed data, $p < 0.05$).

The genotypic, phenotypic and interaction effects on relative dry mass allocation to the different plant parts are shown in Table 2.6. Clonal variation in the proportional allocation to the different organs was significant in all cases. On the other hand, the availability of water had a significant effect only on the allocation to root and branch dry mass, in which case the mean relative dry mass allocation to roots was reduced under low water availability and the mean relative allocation to branch dry mass increased. The only significant interaction effect between clone and water treatment was found in the allocation to main stem dry mass where clones 2 and 4 responded to lower water availability by increasing allocation to this plant part whereas the other clones responded with a reduction which suggests inherent genetic variation in storage patterns under moisture stress.

Table 2.6 Two way analysis of variance listing the effects of clone (n=6) and water treatment (n=2) on relative biomass allocation to plant organs. Analysis was performed on arcsin transformed data. A separate ANOVA was performed for each organ. Values are F values, *significant differences at $p < 0.05$. NS = not significant

Organ	Clonal effect	Treatment	Interaction
Root	12.89 *	21.64 *	1.18 NS
Main stem	17.63 *	1.26 NS	2.96 *
Leaves	30.36 *	3.94 NS	0.39 NS
Side stem	12.54 *	39.40 *	1.75 NS

2.3.7 Growth traits related to the allocation to harvestable stem wood

RGR and the allocation to harvestable stem wood (H) did not correlate significantly amongst individuals of W_h ($p > 0.05$), whereas H increased significantly with RGR under water limited conditions ($p < 0.05$) (Figures 2.12 a - b). The lack of a significant positive correlation between H and RGR in W_h is caused by the clonal variation in dry mass allocation patterns. Significant multiple linear regressions derived from a step-wise regression model (Table 2.7) showed that H increases linearly with simultaneous decreases in branch dry mass and LWR and increases in canopy leaf area in W_h ($r^2 = 0.58$).

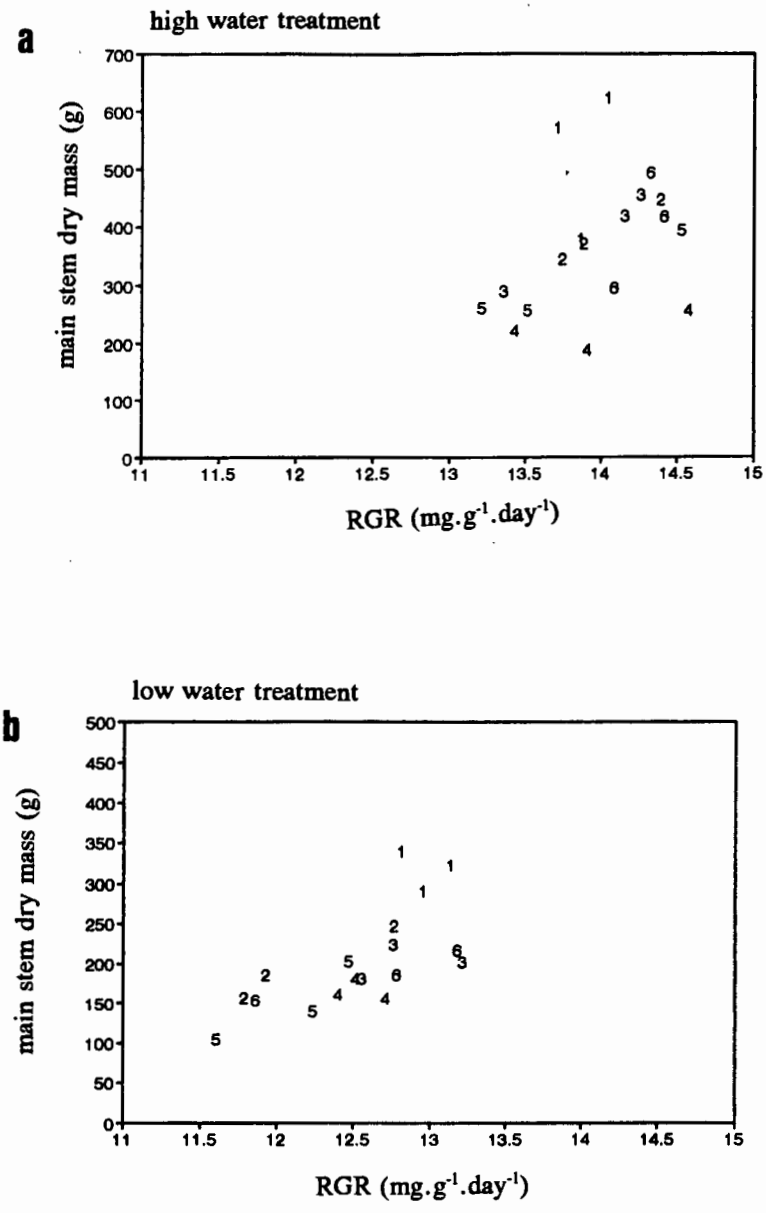


Figure 2.12 Relationship between main stem biomass (H) and RGR for individuals of W_h (a), r = 0.38, $p > 0.05$ and for W_l (b), main stem = 87.05 RGR - 887.68, r = 0.68, $p < 0.05$.

Table 2.7 Stepwise Multiple Regression Model estimation of the significant growth characteristics influencing harvestable wood (H) at $p < 0.05$ of *Eucalyptus* seedlings under high and low water availability. Regression equations and coefficients of determination (r^2) for the multiple effects of the significant variables are given.

Regression equation	r^2
High water treatment	
$H = 554.12 - 0.90\ branch_m$	0.22
$H = 450.78 - 1.33\ branch_m + 101.8$ canopy leaf area	0.40
$H = 517.21 - 1.60\ branch_m + 198.39$ canopy leaf area - 1386.1 LWR	0.58
Low water treatment	
$H = 441.46 - 3.61$ SLA	0.23
$H = 321.60 - 2.98$ SLA $+ 0.15\ root_m$	0.36

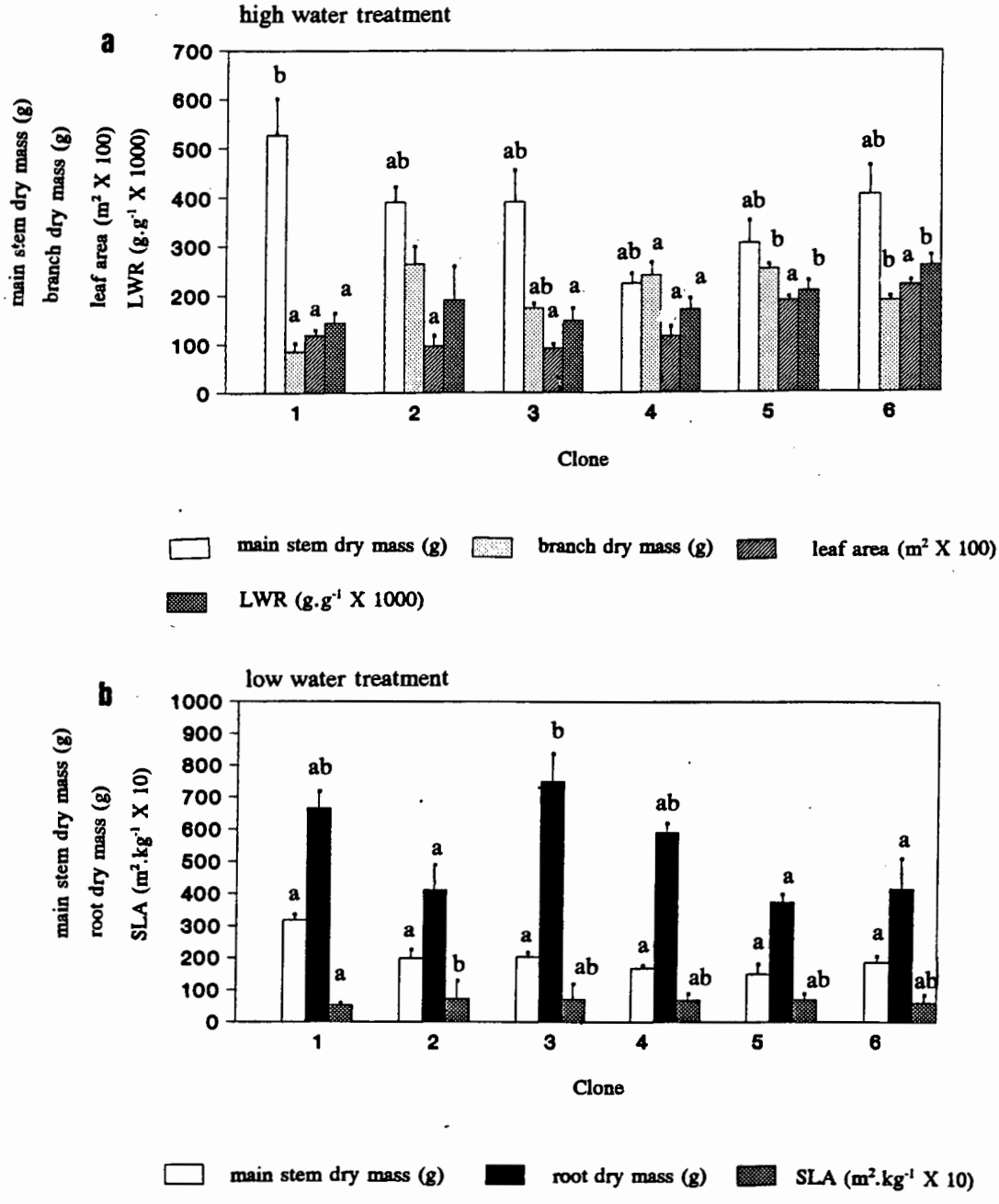


Figure 2.13 (a) Mean clonal variation in main stem biomass, branch biomass, leaf area and leaf weight ratios for individuals of W_h, (b) mean clonal variation in main stem biomass, root biomass and SLA for individuals of W_l. Different letters indicate means are significantly different at $p < 0.05$ (Tukey Multiple Range test).

A comparison made between mean clonal dry mass allocation patterns show that significantly higher values of H in clone 1 coincide with significantly lower branch dry mass whereas the increase in branch dry mass in clones 4 and 5 coincides with a lower investment in H (Figure 13 a). Similarly a significantly lower LWR in clone 1 corresponds with the higher allocation to H in comparison to the lower investment in H with the significantly higher LWR ($F_{5,12}$ = 15.8, $p < 0.05$) in clones 5 and 6. The positive effects of the increase in leaf area on H may be independent of the trade-off with LWR, and the significant effects derived from the model may underlie the positive correlation found between total dry mass accumulation and canopy leaf areas (Figure 2.5 a).

Under water limitation, allocation to H was found to increase linearly with an increase in root dry mass and a decrease in SLA (the production of thicker and smaller leaves) (r^2 = 0.36). This pattern is evident in Figure 2.13 b where the relatively high dry mass allocation to H in clone 1 results mainly from higher root dry mass and lower SLA ($F_{5,12}$ = 3.6, $p <$ 0.05) than clones 2 and 5.

2.4 DISCUSSION

This study confirms that growth of *Eucalyptus* decreases significantly with a reduction in water availability. Growth was found to be inhibited through the reduction in carbon assimilation due to lower stomatal conductance under lower water availability. Individuals of W_h produced higher canopy leaf areas, lower nitrogen concentrations per unit leaf mass, and higher rates of photosynthesis, stomatal conductance and transpiration in the field. Foliar nitrogen concentrations in individuals of both W_h and W_l were not a significant determinant of photosynthetic activity per unit leaf area as was expected from recent work carried out on *E. grandis* seedlings where higher rates of photosynthesis were recorded at higher foliar nitrogen concentrations (Sands et al.,1992). Instead photosynthetic activity per unit leaf area was strongly dependent on stomatal conductance and rates of transpiration which were both strongly influenced by plant water status as affected by soil moisture availability and atmospheric relative humidity.

As was found in previous nutrition and water related studies on species of *Eucalyptus* (Sheriff and Nambiar, 1992, Pereira et al., 1993), increases in RGR were not affected by

photosynthetic activity at the leaf level. Instead it was found that increases in mean RGR were brought about by increases in canopy leaf area in W_h and through decreases in SLA in W_l. This suggests that under adequate water supply net carbon assimilation is maximized by increasing the amount of solar radiation intercepted by the canopy at the expense of greater water loss whereas water limitation causes a reduction in surface leaf area to minimise water loss with the result that photosynthetic apparatus are stacked into denser packages.

Under both high and low water availability, increases in mean RGR were found to be mediated through increases in NAR which corresponded with high root:leaf area ratios (absorption mass:transpiration surface ratio), canopy leaf areas and low specific leaf areas (the production of thick leaves). In addition, under low moisture availability where growth was more strongly dependent on water uptake from the soil, higher mean RGRs were achieved through the reduction in LWR, with the resultant larger ratio of water-supply tissue to water-evaporating tissue. Similarly Mooney et al. (1978) reported that eucalypt species growing in the arid regions of Australia exhibited higher root dry mass, higher root to leaf mass, lower leaf area per length of stem, higher NAR and lower LAR than species originating in coastal moist regions. NAR was found to be the main strategy employed by the plants at both moisture availabilities which implies that this growth trait is genetically determined and suggests the possibility that these six genotypes may have been cloned from provenances originating in arid regions of Australia. Of greater importance, the growth traits exhibited under different moisture regimes in this experiment show that species of *Eucalyptus* are capable of withstanding water shortages mainly by reducing transpiration surfaces and increasing the density of photosynthetic apparatus per unit leaf area (Table 2.4, Figure 2.9 b). With increasing water availability plants respond primarily with the growth of more roots to increase water uptake and more leaves to increase carbon assimilation (Table 2.4, Figures 2.3 a and 2.5 a).

Significant variation in dry mass allocation to root and leaf dry mass as found in this study suggests that NAR and LAR may differ between clones, but the lack of significant differences in mean RGR found among clones indicate that the degree to which NAR and LAR may contribute to RGR is probably counteractive amongst clones. However clonal variation in traits such as root:leaf area, SLA and LWR may have an effect on clonal water

use efficiency which will be investigated in chapter 3.

Clonal variation in dry mass allocation to harvestable wood was found in commercial clones of *Eucalyptus* at 16 months. However the lack of a significant correlation between harvestable stem wood dry mass and RGR under a high soil moisture availability suggests the complexity of processes governing the carbon allocation in eucalypt species. Clonal differentiation in carbon allocation to harvestable stem wood under high water supply was found to be achieved at the expense of investment in branch dry mass and leaves. Under low soil moisture availability, allocation to harvestable stem dry mass was positively correlated with RGR and was strongly influenced by root dry mass and SLA.

This study revealed that genetic variation in dry mass allocation to harvestable stem dry mass was mainly due to a trade-off of carbon allocation to other plant parts. Allocation to harvestable stem dry mass varied amongst clones with changing water availability. Some clones responded with an increase in allocation to this plant part under stress which suggests genetic variation in storage patterns. The genetic and physiological basis for the variation in dry mass allocation to harvestable stem lie beyond the limits of this study. Further exploration of the physiological processes underlying the carbon partitioning to stem dry mass for conductance and storage in *Eucalyptus* should be carried out.

CHAPTER THREE

WATER USE EFFICIENCY IN COMMERCIAL CLONES OF *EUCALYPTUS* IN SOUTH AFRICA

3.1. INTRODUCTION

Productivity in terms of economic yield to both the agriculturalist and forester has been greatly influenced by the availability of water for plant growth. The goal of many plant breeders is to develop cultivars that produce the highest marketable yield under a given moisture regime (Barnes, 1983).

Water use efficiency (WUE) is an index which describes the unit assimilation per unit water loss and has been found to be quite variable in agricultural crop cultivars and genotypes (Cole et al., 1970, Farquhar and Richards, 1984, Martin and Thorstenson, 1988, Vos and Groenwold, 1989). Despite the considerable number of genetic studies that have been directed at improving plant yields and understanding plant water relations, little is known about the genetic basis for the variation in water use efficiency (Barnes, 1983). This lack of information, which is of vital importance to plant breeding programmes is mainly a consequence of the difficulty in analysing the integration of processes regulating water use efficiency at the level of the whole plant. Also measures of water use efficiency are not as simple as those used to determine growth increments. Consequently, there are few empirical studies showing the influence of plant physiological, morphological or anatomical traits on whole plant water use efficiency.

Hypothetical models for the regulation of water use efficiency have been proposed at the level of the leaf (Cowan and Farquhar, 1977, Farquhar et al., 1982, Farquhar and Richards, 1984) and the whole plant (Schulze et al., 1983). Water use efficiency is primarily determined through the ratio of rates of photosynthesis (A) to transpiration (E) in the leaf. Cowan and Farquhar (1977) suggested that A/E is mainly a function of optimal stomatal behaviour in which case stomatal conductance is adjusted so that the total loss of water through transpiration is minimized for the total amount of carbon taken up through photosynthesis per unit time. The underlying assumption of this hypothesis is that the sensitivities of transpiration and CO_2 uptake to changes in stomatal conductance remained constant over time. However it has been acknowledged that optimal stomatal control of A/E as regulated through the feedback response to leaf intercellular CO_2 concentrations and internal water relations may not be the primary determinant of water use efficiency over the life of the plant, as A/E may not remain constant under changing irradiance, air temperature

and atmospheric humidity (Schulze et al., 1983, Farquhar et al., 1988).

Subsequently it has been suggested that variation in water use efficiency may be related to the variation in p_i/p_a (ratios of internal to external concentrations of CO_2) in plants (Farquhar et al., 1982, Farquhar and Richards, 1984, Farquhar et al., 1988). The link between water use efficiency and p_i/p_a is based on the fact that high rates of carbon fixation require that mesophyll CO_2 concentrations are maintained through high rates of stomatal conductance with a consequent high rate of water loss through transpiration, and vice versa. Thus, the link between the abundance ratios of stable carbon isotopes and water use efficiency through variation in p_i/p_a suggested by Farquhar et al. (1982) may provide a useful tool in establishing the genetic basis for variation in water use efficiency (see chapters 4 and 5).

As an alternative approach to the short term influence of leaf physiological traits on water use efficiency, Schulze et al. (1983) suggest that the long term response of plants to soil moisture availability and relative humidity may be reflected at the level of the whole plant through optimal biomass partitioning between leaves and roots without adversely affecting the water status of the plant. This implies that the growth of new leaves should be accompanied by enhanced water uptake to meet the additional evaporative demand created by increased leaf area without decreasing the water status of the plant. Within the context of this hypothesis, optimal water use efficieny may be achieved through the optimal allocation to root and leaf biomass to ensure the maximum carbon gain for a given amount of water loss. Plant traits such as canopy leaf area index (canopy density), specific leaf area, foliar nitrogen per unit area, root:shoot and root:leaf area ratios (absorption to transpiring surface area) are expected to influence the carbon gaining:water losing capacity of a plant.

A hypothetical plant having a dense canopy of leaves with low specific leaf areas and high foliar nitrogen per unit leaf area, as opposed to one characterized by an open canopy, large specific leaf areas and low foliar nitrogen per unit leaf area, should have a higher carbon gaining:water losing capacity at the canopy level for the following reasons:

Dense canopies maintain a higher air humidity within the canopy boundary layer which should result in lower rates of canopy transpiration than open canopies. Furthermore, a

dense canopy of small, thick leaves and high foliar nitrogen per unit area should have a larger carbon gaining:water losing capacity at the individual leaf level and in turn contribute to a higher water use efficiency at the canopy level. It is also expected that higher water use efficiencies could be achieved through low root:shoot and root:leaf area ratios if it is assumed that allocation to root biomass meets the minimal requirement for water uptake so as to maintain a plant water status at which photosynthetic gain to water loss is maximised at the leaf level. It is within the context of this latter hypothesis that this chapter addresses the influence of dry mass allocation and whole plant morphology on water use efficiency in clonal genotypes of *Eucalyptus,* commonly grown in South Africa.

Variation in water use efficiency (m^3 wood/kl water) has been reported for 4 year old clonal genotypes of *Eucalyptus grandis* in South Africa (Olbrich et al. 1993). As yet, the influence of patterns of dry mass allocation and canopy structure and leaf morphology on water use efficiency (in terms of total, shoot or harvestable stem wood dry mass productivity) is unknown. If plant traits that are strongly correlated with water use efficiency can be identified and are genetically fixed, the use of these traits in screening for WUE could be potentially useful to the commercial tree breeder. With this objective in mind, the study was conducted to answer the following questions:

1.(a) Is clonal variation in water use efficiency (total, shoot, main stem) expressed at the juvenile stage (< 2 years) in eucalypt genotypes commonly grown in South Africa?

(b) Is there phenotypic plasticity in water use efficiency in response to different soil moisture availability? If so, are there significant genotypic-phenotypic interactions ?

2. What are the optimal patterns of dry mass allocation, canopy structure and leaf morphology to obtain optimal water use efficiency in commercial clones of *Eucalyptus* commonly grown in South Africa?

3.2. METHODS

3.2.1. Water use efficiency

Total dry mass accumulated and water used in clonal genotypes of eucalyptus were determined in the manner described in chapter 2, sections 2.2.7.2 and 2.2.2.6 respectively. Total plant, shoot and harvestable stem wood water use efficiency were calculated as:

$$WUE_p = \frac{\text{whole plant dry mass (g)}}{\text{water use over growth period (l)}}$$

$$WUE_{sh} = \frac{\text{shoot dry mass (g)}}{\text{water use over growth period (l)}}$$

$$WUE_h = \frac{\text{harvestable stem dry mass (g)}}{\text{water use over growth period (l)}}$$

3.2.2. Variables of dry mass allocation, canopy structure and leaf morphology

Root:shoot, root:leaf area ratios, specific leaf areas, foliar nitrogen per unit leaf area, canopy leaf area index, leaf weight ratios, leaf:stem ratios (canopy leaf weight/canopy stem weight) were determined in the manner described in chapter 2, sections 2.2.7. to 2.2.10.

3.2.3. Statistical analyses

Statistical analyses and graphic display were performed using the STATGRAPHICS (Version 5.0, Statistical Graphics Corp., Maryland, USA) and QUATRO PRO (Version 4.0, Borland International INC., California, USA) software packages.

3.2.3.1 *Parameters describing clonal and treatment effects*

A Two Way Analysis of Variance model was used to determine the effects of water availability and genotype on WUE_p, WUE_{sh} and WUE_h and on the variables describing patterns of dry mass allocation and canopy morphology, after which a Tukey Multiple Range test was used to detect significant differences amongst the means (treatments not separated) (Zar, 1984).

3.2.3.2 *Relationships among variables*

Multiple correlations were used to determine significant correlations amongst WUE_p, WUE_{sh} and WUE_h and dry mass allocation and canopy morphology variables after which Simple

Linear Regression analyses were carried out to establish the relative dependence of the variable in question on another. Difference between regression slopes for the two treatments were tested using Students't test (Zar. 1984). In the case where slopes were not significantly different at $p < 0.05$, Simple Linear Regression analyses were performed on the combined data of both W_h and W_l. Stepwise Linear Multiple Regression models were used to determine the combined effects of significant variables ($p < 0.05$) on WUE_p, WUE_{sh} and WUE_h.

3.3 RESULTS

3.3.1 Clonal variation in water use efficiency

Mean clonal WUE in terms of total plant, shoot and harvestable stem dry mass under high and low soil moisture availability are shown in Table 3.1. WUE_p, WUE_{sh} and WUE_h were significantly higher in individuals of the W_l treatment (Table 3.2). No significant genotypic interactions with soil moisture availability were found in WUE_p, WUE_{sh} and WUE_h (Table 3.2) which indicates that the plant response in the efficiency of water use under different moisture availability were similar amongst clones i.e. an increase in water use efficiencies under a lower soil moisture availability was evident in all of the clones (Table 3.1).

Average clonal WUE was found to be significantly different at the level of whole plant, shoot and harvestable stem dry mass (Table 3.2), where clone 6 had a higher carbon gain per unit water loss relative to the other clones (Figure 3.1). The ranking changes for different measures of assimilation; clone 1 had the lowest WUE_p but the highest WUE_h mainly as a result of the clonal variation in dry mass allocation between roots, shoots and main stems as was shown in chapter 2, section 2.3.5. This shows that estimates of WUE are sensitive to the units used to measure dry mass assimilation.

3.3.2. The influence of dry mass allocation, canopy structure and leaf morphology on WUE_p, WUE_{sh} and WUE_h

No significant relationships were found between WUE_p and root:shoot dry mass ($r = -0.26$, $p > 0.05$), WUE_p and root:leaf area ratios ($r = -0.28$, $p > 0.05$) and WUE_p and SLA ($r = -0.17$, $p > 0.05$) (data not shown). However WUE_p was positively correlated with foliar nitrogen per unit area ($r^2 = 0.16$) and canopy leaf area index ($r^2 = 0.19$)

Table 3.1 Mean WUE_p, WUE_{sh} and WUE_h in clones of *Eucalyptus* after 16 months under high (W_h) and low (W_l) watering treatments. Values are means with SE in parenthesis. Different letters indicate means are significantly different at $p < 0.05$ (Tukey Multiple Range test after Two Way Analysis of Variance).

Clone	WUE_p (g/l)			WUE_{sh} (g/l)			WUE_h (g/l)		
	W_h	W_l	$W_h + W_l$	W_h	W_l	$W_h + W_l$	W_h	W_l	$W_h + W_l$
1	2.63 (0.15)	3.18 (0.19)	2.90a (0.16)	1.56 (0.12)	1.55 (0.07)	1.35ab (0.11)	0.73 (0.09)	0.78 (0.07)	0.75c (0.05)
2	3.13 (0.23)	3.22 (0.08)	3.17ab (0.11)	1.25 (0.15)	1.75 (0.08)	1.50ab (0.13)	0.56 (0.04)	0.72 (0.03)	0.64bc (0.04)
3	2.83 (0.13)	3.24 (0.11)	3.04ab (0.12)	1.03 (0.04)	1.27 (0.11)	1.15a (0.06)	0.53 (0.02)	0.54 (0.03)	0.53ab (0.02)
4	2.88 (0.31)	3.31 (0.22)	3.10ab (0.20)	0.96 (0.09)	1.45 (0.11)	1.21ab (0.13)	0.31 (0.05)	0.52 (0.02)	0.42a (0.05)
5	2.55 (0.09)	3.35 (0.24)	2.95ab (0.21)	1.28 (0.05)	1.88 (0.28)	1.58bc (0.18)	0.43 (0.03)	0.58 (0.08)	0.51ab (0.05)
6	3.32 (0.45)	3.93 (0.09)	3.62b (0.25)	1.70 (0.18)	2.28 (0.19)	1.99c (0.18)	0.65 (0.05)	0.76 (0.02)	0.70c (0.03)
mean (se)	2.89[a] (0.11)	3.37[b] (0.08)	----- -----	1.23[a] (0.07)	1.70[b] (0.09)	----- -----	0.53[a] (0.04)	0.65[b] (0.03)	----- -----

Table 3.2. F values derived from Two Way Analysis of Variance testing the significance of clone (n=6) and water treatment (n=2) on WUE_p, WUE_{sh} and WUE_h. *significant differences at $p < 0.05$.

WUE	Clonal effect	Treatment	Interaction
WUE_p	2.841*	14.548*	0.608^{NS}
WUE_{sh}	10.318*	36.005*	0.503^{NS}
WUE_h	14.495*	17.688*	1.157^{NS}

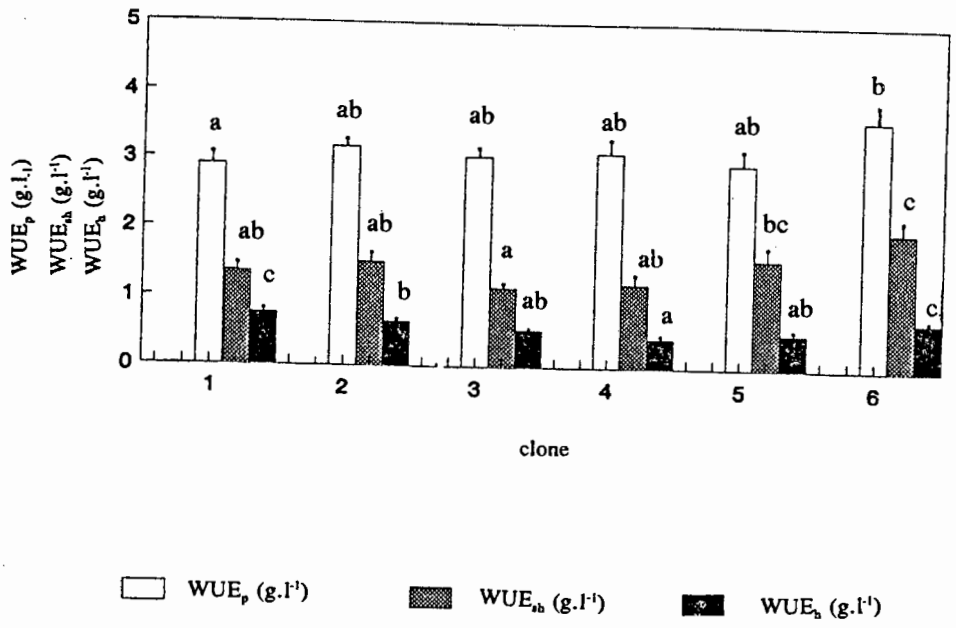


Figure 3.1 Mean clonal WUE_p, WUE_{sh} and WUE_h for 16 month eucalypt seedlings. Different letters indicate significant differences among clones (W_h + W_l) (Tukey Multiple Range with significance at $p < 0.05$).

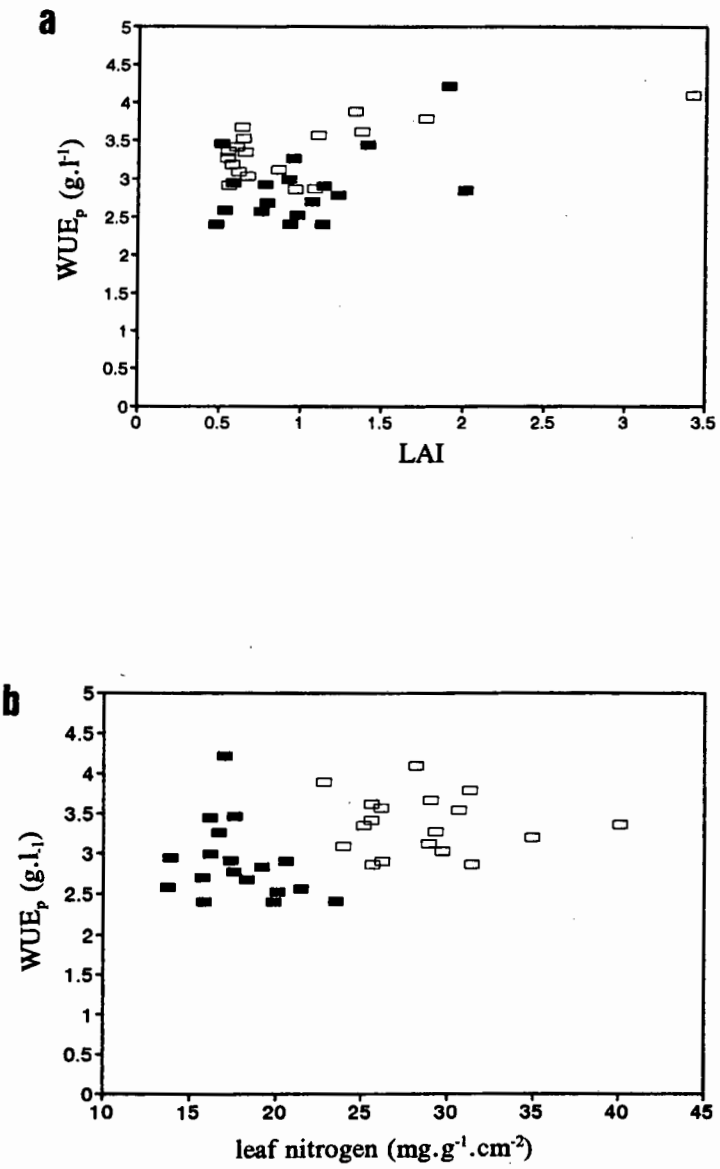


Figure 3.2 (a) Relationship between WUE_p and LAI. No significant differences between regression slopes of individuals in W_h ■ and W_l □ ($t_{1,32} = 0.59$, $p > 0.05$). $WUE_p = 0.37LAI + 2.76$, $r = 0.43$, $p < 0.05$. (b) Relationship between WUE_p and foliar nitrogen per unit area. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 0.41$, $p > 0.05$). $WUE_p = 1.73\ nitrogen_{area} + 2.49$, $r = 0.39$, $p < 0.05$.

(Figures 3.2 a and b). A Stepwise linear regression model estimate (Table 3.3) showed that only 27% of the variation in WUE_p of the experimental plants could be explained by the effects of increases in foliar nitrogen per unit area and canopy leaf area index. However it was found that the significantly higher WUE_p of clone 6 coincided with significantly higher canopy leaf area index ($F_{5,1} = 8.9$, $p < 0.05$) and foliar nitrogen per unit leaf area ($F_{5,1} = 3.7$, $p < 0.05$) (Figure 3.3).

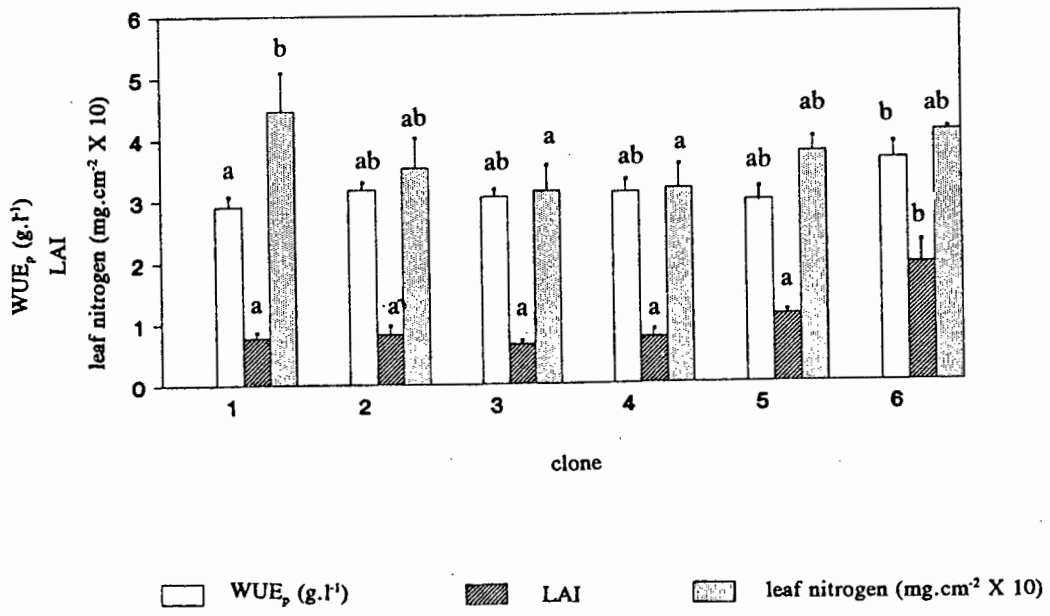


Figure 3.3 Mean clonal WUE_p, LAI and foliar nitrogen per unit area. Different letters indicate significant differences among clones ($W_h + W_l$) (Tukey multiple range with significance at $p < 0.05$).

Increases in WUE_{sh} correlated significantly with increases in leaf weight ratio (LWR), leaf:stem ratios, foliar nitrogen per unit leaf area and canopy leaf area index and a decrease in root:leaf area ratio (Figures 3.4 a - e). Significant multiple regressions derived from a Stepwise regression model showed that 71 % of the variation in WUE_{sh} could be explained through the effects of increases in foliar nitrogen per unit leaf area, decreases in root:leaf area ratios and increases in canopy LAI (Table 3.3). Furthermore the significantly higher canopy LAI and significantly lower root:leaf area ratios ($F_{5,1} = 8.4$, $p < 0.05$) coincided with the significantly higher WUE_{sh} of clone 6 relative to clones 3 and 4 (Figure 3.5).

WUE_h was found to increase significantly with increases in foliar nitrogen per unit area and decreases in specific leaf area (Figures 3.6 a and b). Decreases in root:leaf area and root:shoot ratios was found to be significantly correlated with increases in WUE_h (Figures 3.6 c and d) .

Table 3.3 Stepwise multiple regression model estimation of the significant plant structural and morphological traits influencing WUE_p and WUE_{sh} at $p < 0.05$ of eucalypt seedlings. Regression equations and coefficients of determination, r^2 values, for the effects of the significant variables are given. Watering treatments were not separated in regression analyses.

Regression	r^2
$WUE_p = 0.366\ LAI + 2.76$	0.17
$WUE_p = 0.027\ nitrogen_{area} + 0.378\ LAI$	0.27
$WUE_{sh} = 2.104\ nitrogen_{area} + 0.688$	0.27
$WUE_{sh} = 1.263\ nitrogen_{area} - 0.001\ root{:}leaf\ area + 1.601$	0.63
$WUE_{sh} = 1.427\ nitrogen_{area} - 0.0006\ root{:}leaf\ area + 0.263\ LAI$	0.71

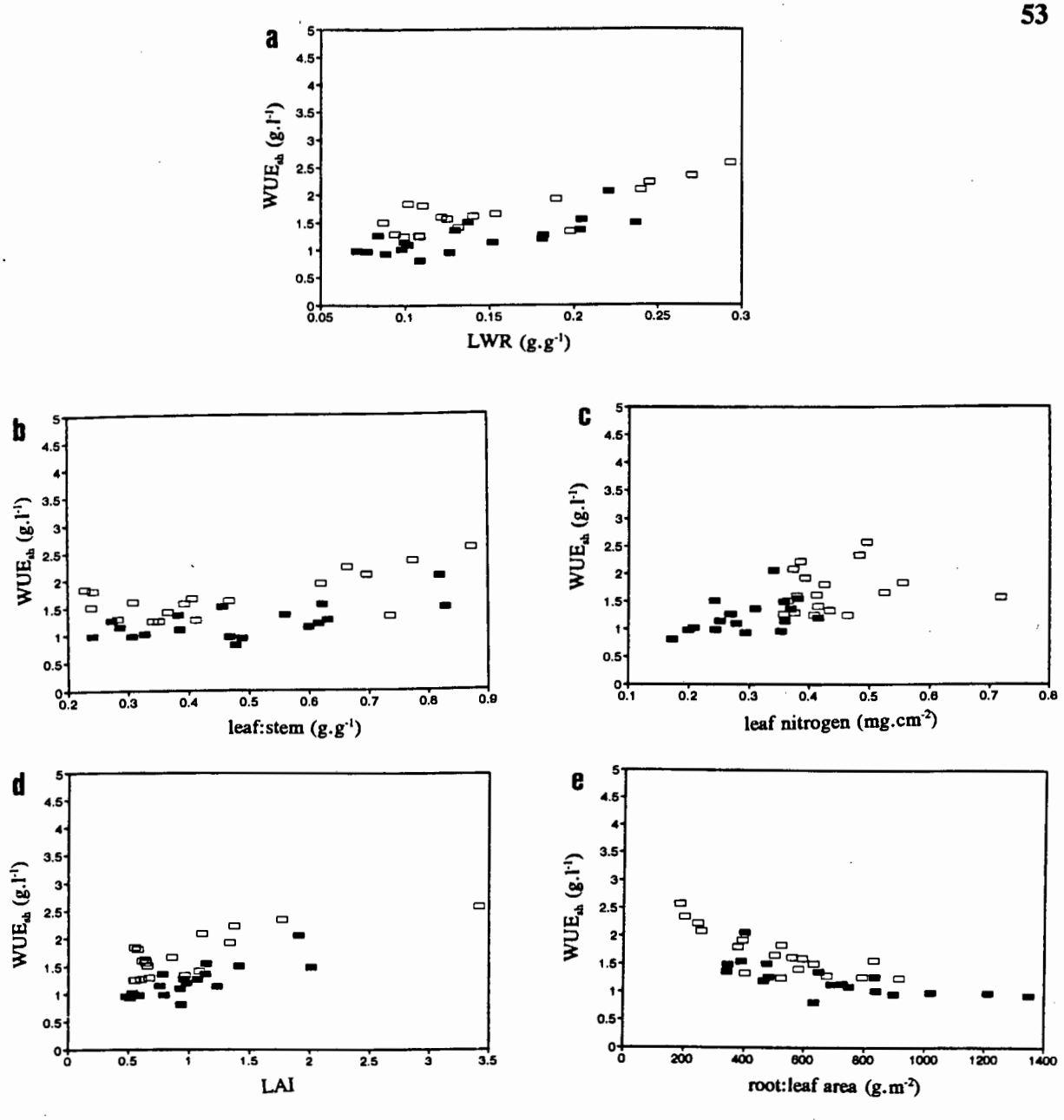


Figure 3.4 (a) Relationship between WUE_{sh} and leaf weight ratio (LWR). No significant differences between regression slopes of individuals in W_h ■ and W_l □ ($t_{1,32} = 0.22$, $p > 0.05$). $WUE_{sh} = 5.18$ LWR + 0.70, $r = 0.73$, $p < 0.05$. (b) Relationship between WUE_{sh} and leaf:stem ratio. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 0.69$, $p > 0.05$). $WUE_{sh} = 1.13 + 0.92$ leaf:stem, $r = 0.50$, $p < 0.05$. (c) Relationship between WUE_{sh} and foliar nitrogen per unit area. No significant relationship between regression slopes of individuals in W_h and W_l ($t_{1,32} = 1.04$, $p > 0.05$). $WUE_{sh} = 2.10$ $nitrogen_{area} + 0.69$, $r = 0.54$, $p < 0.05$. (d) Relationship between WUE_{sh} and canopy leaf area index (LAI). No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 0.68$, $p > 0.05$). $WUE_{sh} = 0.47$ LAI + 0.99, $r = 0.63$, $p < 0.05$. (e) Relationship between WUE_{sh} and root:leaf area ratio. Regression slopes of individuals in W_h and W_l ($t_{1,32} = 2.56$, $p < 0.05$). In W_h, $WUE_{sh} = 1.71 - 0.0007$ root:leaf area, $r = -0.68$, $p < 0.05$. In W_l, $WUE_{sh} = 2.48 - 1.54$ root:leaf area, $r = -0.81$, $p < 0.05$.

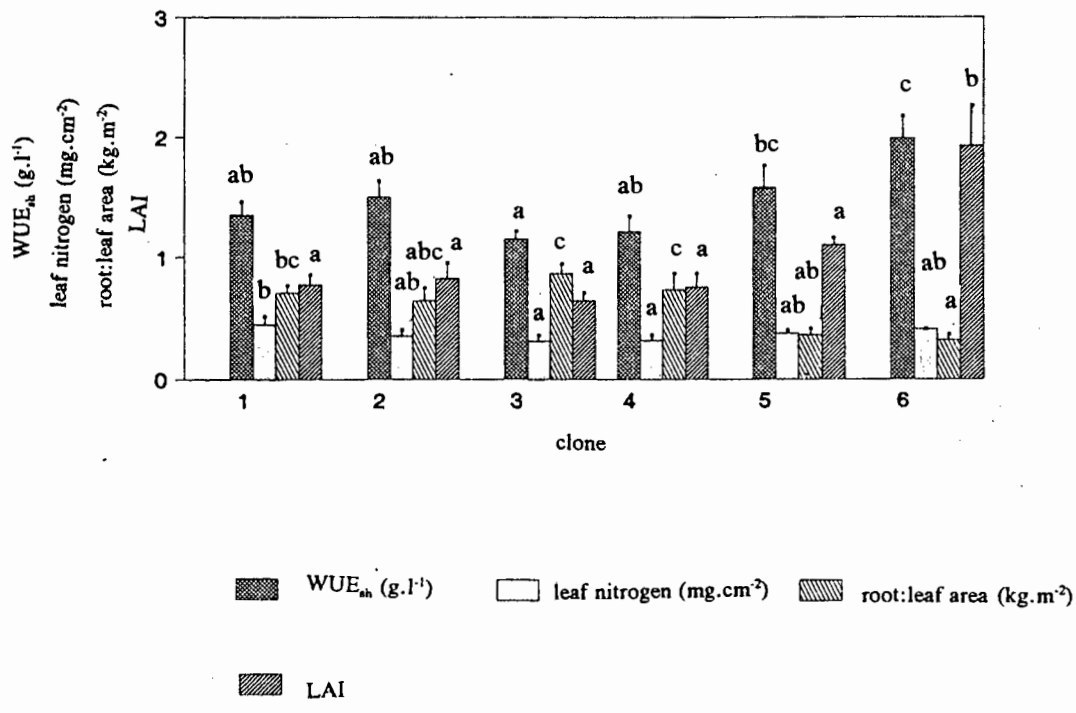


Figure 3.5 Mean clonal WUE_{sh}, foliar nitrogen per unit area, root:leaf area ratios and canopy LAI. Different letters indicate significant differences among clones (W_h +W_l) (Tukey Multiple Range with signficance at $p < 0.05$).

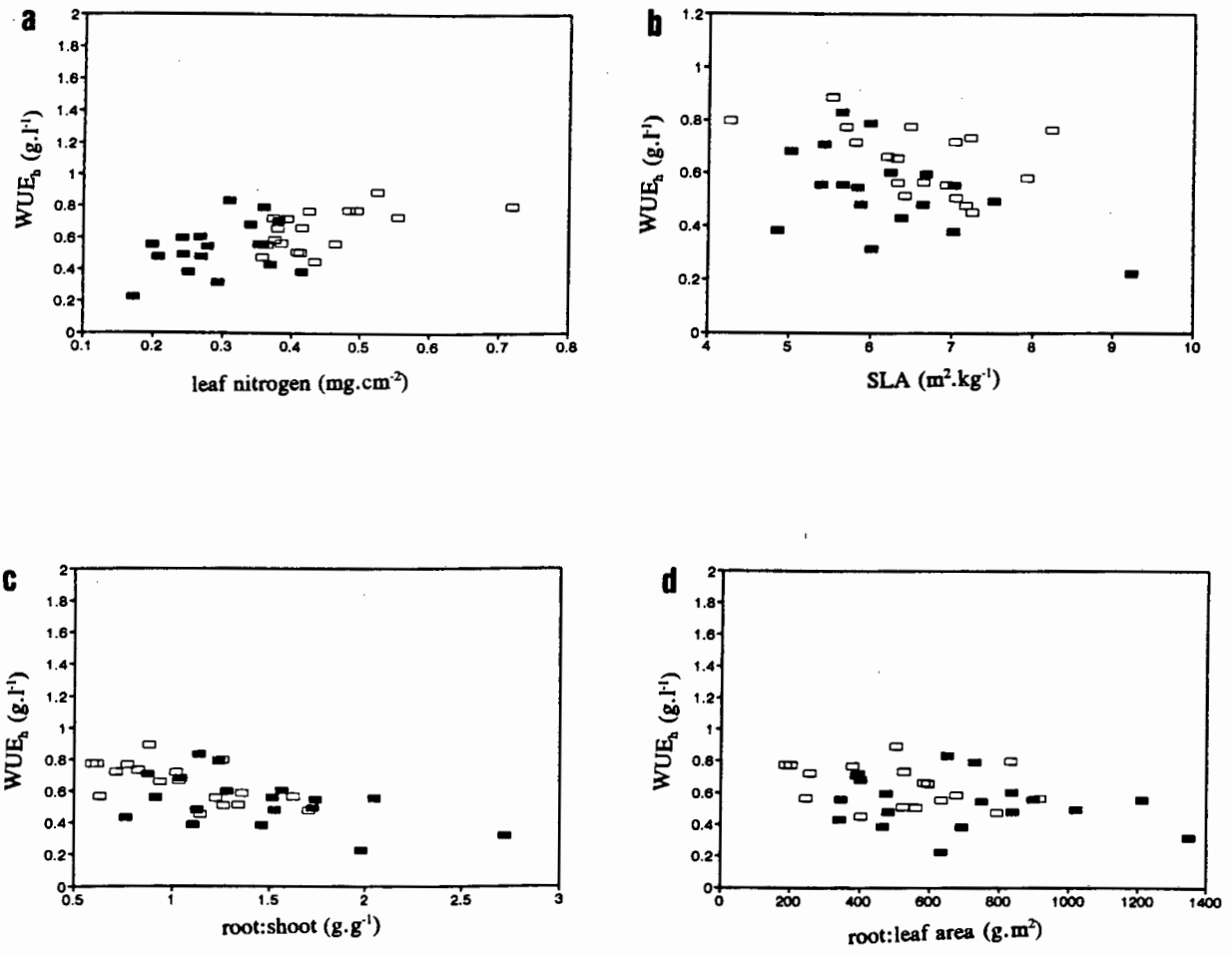


Figure 3.6 (a) Relationship between WUE_h and foliar nitrogen per unit area. No significant differences between regression slopes of individuals of W_h ■ and W_l □ ($t_{1,32}$ = 0.008, $p > 0.05$). WUE_h = 0.80 $nitrogen_{area}$ + 0.30, $r = 0.57$, $p < 0.05$. (b) Relationship between WUE_h and SLA. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32}$ = 0.37, $p > 0.05$). WUE_h = 0.97 - 0.006 SLA, $r = -0.37$, $p < 0.05$. (c) Relationship between WUE_h and root:shoot ratio. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32}$ = 0.74, $p > 0.05$). WUE_h = 0.84 - 0.20 root:shoot, $r = -0.60$, $p < 0.05$. (d) Relationship between WUE_h and root:leaf area. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32}$ = 0.36, $p > 0.05$). WUE_h = 0.71 - 0.0002 root:leaf area, $r = -0.34$, $p < 0.05$.

3.4 DISCUSSION

Clonal variation in WUE is expressed within the first 2 years of the growth period of clonal genotypes commonly grown in South Africa (Table 3.1). As was found in other studies on WUE in tomato cultivars by Martin and Thorstenson (1988) and crested wheatgrass by Read et al. (1991), WUE increased under conditions of low soil moisture availability in the clonal genotypes of *Eucalyptus* investigated in this study. Clonal responses in water use efficiency to different soil moisture availability were similar with respect to whole plant, shoot and harvestable dry mass.

Variation in WUE_p, WUE_{sh} and WUE_h were found to be influenced by patterns of dry mass allocation and canopy morphology. Dense leaf canopies and high nitrogen per unit leaf area were found to enhance WUE_p significantly. However the combination of these two factors were only able to explain 27% of the variation in WUE_p. Unexplained variation in WUE_p may be influenced by the variation in instantaneous water use efficiencies at the leaf level within the same canopy due to differences in leaf age and position in the canopy (Wullschleger and Oosterhuis, 1989).

Clonal variation in WUE_{sh} and WUE_h was strongly influenced by the relative proportions of dry mass allocated to shoot and harvestable stem wood dry mass. It was found that high WUE's were derived mainly through reduced dry mass allocation to roots with little change in investment to leaves and stem dry mass. Consequently the clonal ranking of WUE_p is not always the same as the clonal ranking for the expenditure of water per unit carbon gain of harvestable dry mass.

Root:leaf area ratios together with dense canopies with high nitrogen per unit leaf area and low specific leaf areas were shown to contribute significantly to high WUE_{sh} and WUE_h in 16 month old eucalypt clones investigated in this study. Even though the relative patterns of dry mass allocation to roots, stems and leaves may differ between the seedling and mature stage of tree development, the hypothetical concept that optimal WUE may be achieved through the optimal allocation to roots, stems and leaves without adversely affecting the water status of the plant is possible. However the optimal allocation patterns to root, stem and leaf dry mass to obtain maximum WUE in adult trees may be different to that required

at the juvenile stage of development. Similarly leaf to stem ratios, canopy leaf area index and specific leaf areas are expected to change with the development of the tree. Due to the practical difficulty associated with working with older (> 4 years) trees of *Eucalyptus*, it would be difficult to establish relationships between traits such as root:leaf area ratios, leaf:stem ratios and leaf area index between young and older trees. The effects of leaf level physiological factors determining p_i/p_a ratios may be of greater importance and of higher practical value in establishing genetic variation in water use efficiency.

This study has shown that clonal variation in WUE is expressed within the first two years of planting in eucalypt genotypes commonly grown in South Africa. Significant increases in WUE under lower soil moisture availability were found in all 6 clones. Variation in WUE was found to be influenced by the allocation of dry mass to roots, stems and leaves. Variation in plant traits such as dense leaf canopies and high nitrogen per unit leaf area contributing to lower specific leaf areas were found to have a significant influence on the WUE of the eucalypt clones investigated in this study. However the influence of these traits on clonal WUE's in mature trees remains questionable and are suggested to hold little potential value as useful criteria for the selection for WUE in *Eucalyptus* commonly grown in South Africa.

CHAPTER FOUR

STABLE CARBON ISOTOPE DISTRIBUTION AMONG PLANT TISSUES AND LEAF ORGANIC COMPOUNDS WITHIN A EUCALYPT CANOPY

4.1 INTRODUCTION

It has been established that land plants are depleted in ^{13}C relative to atmospheric CO_2 and carbonates and that the level of depletion is related to the C_3 and C_4 photosynthetic carbon reduction pathways (Nier and Gulbransen, 1939, Bender, 1968, Troughton et al., 1974, Vogel, 1980). The range of isotopic ratios or $\delta^{13}C$ values (carbon isotope ratios of samples expressed against the known ratios of the Pee Dee Belemnite (PDB) limestone standard) typical for C_3 and C_4 plants are -36 to -21 $^0/_{00}$ and -16 to -8$^0/_{00}$ respectively (Troughton et al., 1974, Vogel, 1980), while mean atmospheric $\delta^{13}C$ values are approximately -7.8$^0/_{00}$ (Keeling, 1979).

Work carried out during the past two decades suggests that the theoretical basis for isotopic fractionation between ^{13}C and ^{12}C during carbon dioxide uptake and fixation by leaves is directly linked to equilibrium and kinetic effects operating on differences in isotopic masses (Vogel, 1980, O'Leary, 1981, Farquhar et al., 1982). Consequently fractionation is associated with the relative magnitudes of the diffusional resistance and the resistance of the carboxylation reaction to the flux of carbon dioxide into the plant.

Farquhar et al. (1982) formulated the relative effects of diffusion and carboxylation resistances on the resultant carbon isotopic ratios of plants relative to PDB in the following equation:

$$\delta^{13}C_{pl} = \delta^{13}C_{atm} - a - (b-a)p_i/p_a, \quad (4.1)$$

where $\delta^{13}C_{pl}$ and $\delta^{13}C_{atm}$ are the isotopic ratios of plant and atmosphere, respectively; a = discrimination against ^{13}C associated with stomatal diffusion, 4.4$^0/_{00}$, b = discrimination against ^{13}C associated with carboxylation, 30$^0/_{00}$ and p_i/p_a is the ratio of intercellular and atmospheric partial pressures of CO_2. Mean $\delta^{13}C$ values obtained for C_3 plants of approximately -28 to -26$^0/_{00}$ suggests that the principal fractionation takes place during carboxylation by RuBP carboxylase (O'Leary, 1981) and that the fractionation is related to p_i/p_a (Farquhar et al., 1982). On the other hand, mean $\delta^{13}C$ values obtained for C_4 plants of approximately -14 to -12$^0/_{00}$ suggests that the principal fractionation takes place during diffusion of CO_2 into the leaf and subsequent fractionation associated with carboxylation by PEP carboxylase is negligible (O'Leary, 1981, Farquhar et al., 1982). Since $\delta^{13}C$ should be linked to p_i/p_a in C_3 plants, the carbon isotopic ratio of a plant tissue should provide an

averaged integral measure of p_i/p_a over time and therefore any physiological processes dependent on this ratio e.g. carboxylation and stomatal conductance (Farquhar and Richards, 1984).

Research during the past decade on variation in $\delta^{13}C$ in C_3 plants has shown that natural variation in carbon isotopic composition of plant tissues correlates well with environmental factors that influence p_i/p_a, namely, irradiance (Ehleringer et al., 1986, Gebauer and Schulze 1991, Medina et al., 1991, Pearcy and Pfitsch, 1991), water stress (Farquhar and Richards, 1984, Hubick et al., 1986, Martin and Thorstenson, 1988), nutrients (Toft et al., 1989) and temperature (Smith et al., 1976). However it has also been shown that $\delta^{13}C$ values in C_3 plants may be influenced substantially by the carbon isotopic composition of source carbon in closed canopies (Vogel, 1978, Medina and Minchin, 1980, Schlesser and Jayasekera, 1985, Medina et al., 1986, van der Merwe and Medina, 1989) and by any fractionation associated with the metabolism of organic compounds succeeding the primary fractionation during carboxylation (Park and Epstein, 1961, De Niro and Epstein, 1977, Benner et al., 1977). Medina et al. (1986) reported $\delta^{13}C$ values for recycled respiratory CO_2 immediately above the forest floor of -16 $^0/_{00}$ which could account for values of -36 $^0/_{00}$ recorded for plants in this habitat. Park and Epstein (1961) reported that lipids can be depleted by as much as 8 $^0/_{00}$ due to fractionation associated with the pyruvate dehydrogenase reaction step (De Niro and Epstein, 1977). This implies that the use of $\delta^{13}C$ in tissues as an integral measure of any physiological process associated with p_i/p_a could be masked by fractionation steps not directly associated with p_i/p_a and carboxylation discrimination.

Studies have shown that $\delta^{13}C$ values of tissues also vary within an individual canopy (Schleser, 1990, Wullschleger and Oosterhuis, 1989, Schleser, 1992). These differences have been attributed to the influence of microenvironmental factors (i.e. irradiance and relative humidity) on the physiological status of leaves (Francey et al., 1985, Wullschleger and Oosterhuis, 1989) as well as the tissue composition of differently labelled organic compounds due to fractionation associated with metabolism and translocation processes (Schleser 1990, Schleser, 1992). Therefore the different causes of natural variation in stable carbon isotope composition in a plant canopy need to be considered when choosing tissue position, tissue type and organic compounds for the inference of physiological information such as plant

water use efficiency from stable carbon isotope ratios in plant tissues.

In this chapter, the natural variation of $\delta^{13}C$ in a eucalypt canopy was explored amongst canopy tissues (leaves and twigs) and organic constituents (soluble sugars, amino acids and phenolics; lipids; starch and crude wall fibre). The aim was to develop an appropriate sampling strategy for isotope analyses of canopy tissues needed in order to verify the relationship between $\delta^{13}C$ and water use efficiency in eucalypt trees (Chapter 5). With this objective in mind, the following questions were addressed:

1. How much variation exists in $\delta^{13}C$ between young leaf, mature leaf and twig tissue?
2. How much variation exists in $\delta^{13}C$ between of foliage and twig tissue sampled at different positions (i.e. aspects) in a eucalypt canopy?
3. How much variation exists in $\delta^{13}C$ between different organic constituents within a eucalypt leaf?
4. Are $\delta^{13}C$ values of plant tissues and organic constituents correlated or does one have to separate constituents to improve physiological information ?

4.2 METHODS

4.2.1 Study site and sampling procedure

Foliage and twig samples were collected from 12 four year old *Eucalyptus grandis* trees (± 23 m high) immediately after felling during April/May 1991 at the Frankfort Forestry Station near Sabie, Eastern Transvaal (25°3'10"S, 30°53'30"E). 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 on north and south sides. Appoximately 100 leaves and five supporting twigs were collected from the respective positions. After collection samples were dried at 70°C and ground in a Wiley mill to a 60 mesh size.

4.2.2 Extraction of leaf organic constituents

Chemical extraction of organic constituents from leaves were carried out in the Botany department of the University of Cape Town.

Four leaf organic categories (soluble carbohydrates, amino acids and phenolics; lipids; starch and crude wall fibre) were extracted from mature leaf tissue from the north aspect by a

procedure derived from a combination of methods outlined by Schlesinger and Hasey (1981) and Brugnoli et al. (1988). One gram of finely ground leaf tissue and 50ml of 80% (v/v) ethanol were heated in Erhlenmeyer flasks at 70°C in a water bath for 60 minutes. Samples were shaken for approximately two minutes at ten minute intervals. Samples were centrifuged at 2800 rpm for 4 minutes after which the ethanol extracts containing soluble carbohydrates, amino acids and phenolics (soluble organics) were decanted and stored in Erlenmeyer flasks. Equal volumes (50 ml) of petroleum ether and 6% (w/w) HCl were added to the remaining plant residues to solubize lipids and starch respectively. Samples were poured into plastic bottles and were allowed to stand for twenty four hours with intermittent shaking. They were subsequently placed in a freezer (-18°C) for 12 hours to separate the liquid petroleum ether from the frozen 6% (w/w) HCl extract. After 12 hours of freezing, the liquid petroleum ether extracts were decanted and stored. HCl extracts and remaining crude wall fibre residue were allowed to defrost and were separated by filtration through Whatman glass microfibre filters using a Buchner funnel. The 6% (w/w) HCl extracts were collected and transferred to Erhlenmeyer flasks. The remaining crude wall fibre residues were rinsed three times with distilled water after which the residue was scraped off the filters with a steel spatula and placed into vials and oven dried at 70°C for 72 hours. The ethanol, petroleum ether and 6% (w/w) HCl extracts were evaporated down from 50 ml to approximately 5 ml by placing the extracts in Erhlenmeyer flasks on a hot plate at 30°C. A gentle air flow was directed into the flasks an air manifold. One ml of the concentrated extracts was placed into quartz micro test tubes (6mm wide and 40mm long) and dried in an oven at 105°C for 4 days. Dry precipitates varyied between 3 and 10 mg.

4.2.3 **Analyses of stable carbon isotopes**

Isotope analyses of the tissue and organic constituents were carried out in the Archeometry laboratory of the University of Cape Town.

4.2.3.1 *Gas production and purification*

Approximately 5 mg of dry plant tissue or 3 to 10 mg dry organic precipitate were loaded approximately 0.5 g of copper oxide and pure copper, and a twist of silver wire into 9 mm quartz tubes. The sample tubes were evacuated to less than 1.33 Pa, sealed with a glassblower's torch and combusted at 800°C in a furnace for 6 h and allowed to cool down

in the furnace for approximately 15 h (Sofer, 1980, Sealy, 1986).

During combustion and cooling, chemical reactions occurring between the organic material, reducing agent (copper oxide) and catalyst (silver) results mainly in the formation of carbon dioxide, copper sulphate, sulphur dioxide, water vapour and nitrogen gas (Sofer, 1980, Sealy, 1986). After cooling the sample tubes were removed from the furnace, scored in the middle, loaded into a cracker and attached to a stainless steel gas-separation line. The whole line was then evacuated to less than 10^{-4} Torr. Gases were released into the evacuated line by cracking the sample. Carbon dioxide and water vapour were frozen in a cold trap for eight minutes with liquid nitrogen. Nitrogen and other gases were pumped out of the line. Carbon dioxide was cryogenically separated from the water vapour by warming the cold trap to a temperature where the carbon dioxide was released into the line in a gaseous phase while the water vapour remained frozen. Carbon dioxide was collected by freezing with liquid nitrogen into a pyrex tube. The collecting tube was sealed off under vacuum using a glassblower's torch.

4.2.3.2 *Measurement of carbon isotope ratios*

Carbon isotope ratios of the carbon dioxide samples were measured on a Micromass 602E dual inlet, double collector mass spectrometer. Carbon dioxide was injected into the mass spectrometer and isotopic ratios were measured relative to a reference gas calibrated against six National Bureau of Standards isotopic reference materials, NBS 16, 17, 18, 19, 20 and 21. The ratio of carbon isotopes were determined as $\delta^{13}C$ calculated relative to the Chicago PDB (Pee Dee Belemnite) marine limestone standard, where

$\delta^{13}C$ = [(R sample/R standard) - 1] X 1000, and R = $^{13}C/^{12}C$. The $\delta^{13}C$ values are given as parts per mil ($^0/_{00}$). A more negative $\delta^{13}C$ means less ^{13}C, a more positive $\delta^{13}C$ means more ^{13}C. A reproducibility of $0.2^0/_{00}$ was obtained on homogenized samples of glucose standards ($-31.8^0/_{00} \pm 0.2$).

4.2.4 **Statistical analyses**

Statistical analyses, data manipulation and graphic display were performed using STATGRAPHICS (Version 5.0, Statistical Graphics Corp., Maryland, USA) and QUATRO PRO (Version 4.0, Borland International INC., California, USA) software packages.

4.2.4.1 *Variances in $\delta^{13}C$ among tissues and organic constituents*

Variances in $\delta^{13}C$ between tissues and organic constituents were analyzed using One Way Analysis of Variance. Differences among means were tested using Tukey Multiple Range test.

4.2.4.2 *Relationships among variables*

Multivariate Correlation analyses were used to determine correlations between $\delta^{13}C$ of tissues and leaf organic constituents. Linear Regression analyses were determined on significant correlations at $p < 0.05$.

4.3 RESULTS

4.3.1 Variation in $\delta^{13}C$ among tissues within a eucalypt tree canopy

The $\delta^{13}C$ values of tissues sampled from within a eucalypt canopy are given in Table 4.1. A range of 1.74 $^0/_{00}$ was found in the mean $\delta^{13}C$ values of canopy tissues. Variation in $\delta^{13}C$ values was greatest in the young leaf tissue, -27.14 $^0/_{00}$ ($\pm$ 0.84) compared to twigs north, -27.23 $^0/_{00}$ ($\pm$ 0.78), mature leaves south, -28.97 $^0/_{00}$ ($\pm$ 0.56), twigs south, -27.46 $^0/_{00}$ ($\pm$ 0.49) and mature leaves north, -28.81 $^0/_{00}$ ($\pm$ 0.34). Young leaf and average twig tissues were found to be significantly more enriched in $\delta^{13}C$ by 1.75 and 1.55 $^0/_{00}$ respectively, relative to averaged whole leaf tissue.

Despite the slight depletion in $\delta^{13}C$ of tissues sampled from the south relative to north aspects of the canopy, no significant differences ($p > 0.05$) were found among mean $\delta^{13}C$ values of twig and mature leaf tissues between north and south aspects.

4.3.2 Variation in $\delta^{13}C$ among leaf organic constituents

Carbon isotopic composition of leaf organic constituents and whole leaf tissue from which they were extracted are presented in Table 4.2. A range of 5.61$^0/_{00}$ was found among $\delta^{13}C$ values of organic constituents within eucalypt leaves. Variation in $\delta^{13}C$ was greatest in the starch component, -25.95$^0/_{00}$ ($\pm$ 0.92) compared to crude wall fibre, -28.01 $^0/_{00}$ ($\pm$ 0.68), soluble organics (sugars, amino acids and phenolics) -30.02 $^0/_{00}$ ($\pm$ 0.62) and lipids, -31.56 $^0/_{00}$ ($\pm$ 0.42).

Mean $\delta^{13}C$ values were significantly different ($p < 0.05$) among organic constituents and whole leaf tissue. Both the soluble organic and lipid components were depleted in ^{13}C relative to whole leaf tissue by 1.24 and 2.78 $^{0}/_{00}$, whereas crude wall fibre and starch were enriched in ^{13}C by 0.77 and 2.86 $^{0}/_{00}$ respectively.

Table 4.1 $\delta^{13}C$ values of tissues sampled from twelve *E. grandis* trees. Differences in $\delta^{13}C$ among tissues are significant ($F_{4,55} = 31.7$, $p < 0.05$). Different letters indicate means are significantly different at $p < 0.05$ (Tukey Multiple Range test)

Tree #	Twigs		Mature leaves		Young leaves
	North	South	North	South	
1	-26.55	-27.35	-28.80	-29.73	-27.65
2	-26.55	-26.66	-28.69	-29.00	-26.64
3	-27.58	-27.63	-29.09	-29.09	-25.22
4	-26.65	-27.73	-28.43	-29.12	-27.09
5	-26.79	-27.07	-28.81	-28.29	-27.09
6	-27.15	-27.59	-28.43	-28.93	-26.41
7	-26.63	-26.68	-29.22	-28.15	-27.60
8	-26.56	-28.05	-28.50	-29.77	-28.03
9	-27.40	-27.68	-28.82	-28.42	-27.67
10	-27.55	-28.17	-28.41	-28.99	-26.66
11	-28.99	-27.13	-29.05	-28.45	-28.33
12	-28.30	-27.81	-29.44	-29.66	-27.34
mean	-27.23a	-27.46a	-28.81b	-28.97b	-27.14a
sd	0.78	0.49	0.34	0.56	0.84
minimum	-26.55	-26.66	-28.41	-28.15	-25.22
maximum	-28.99	-28.17	-29.44	-29.77	-28.33
n	12	12	12	12	12

Table 4.2 $\delta^{13}C$ values of organic constituents extracted from mature leaf tissue from the north aspect from twelve sample trees. Differences in $\delta^{13}C$ among organic constituents and whole leaf tissue are significant ($F_{3,44} = 135.3$, $p < 0.05$). Different letters indicate means are significantly different at $p < 0.05$ (Tukey Multiple Range test)

Tree #	Whole leaf tissue	Crude wall fibre	Starch	Soluble organics*	Lipids
1	-28.80	-27.82	-26.26	-30.25	-32.15
2	-28.69	-27.46	-26.10	-30.95	-31.61
3	-28.78	-28.32	-26.83	-30.41	-31.68
4	-28.43	-27.34	-25.81	-29.95	-30.99
5	-28.81	-27.32	-25.29	-29.97	-31.08
6	-28.43	-28.49	-25.69	-29.32	-31.39
7	-29.22	-27.88	-26.96	-30.43	-32.09
8	-28.50	-28.63	-23.54	-29.18	-31.54
9	-28.82	-27.23	-25.77	-28.91	-31.34
10	-28.41	-27.50	-25.84	-29.85	-31.19
11	-29.05	-28.93	-26.83	-30.43	-31.38
12	-29.44	-29.17	-26.49	-30.61	-32.26
mean	-28.78a	-28.01b	-25.95c	-30.02d	-31.56e
sd	0.33	0.68	0.92	0.62	0.42
minimum	-28.41	-27.23	-23.54	-28.91	-30.99
maximum	-29.44	-29.17	-26.96	-30.95	-32.26
n	12	12	12	12	12

Soluble organics* = soluble sugars, amino acids and phenolics

4.3.3 Correlations between $\delta^{13}C$ of plant tissues and organic constituents

4.3.3.1 Relationships amongst $\delta^{13}C$ of plant tissues

Despite the similarity in the $\delta^{13}C$ values among tissues sampled from north and south aspects, $\delta^{13}C$ values do not correlate significantly ($p > 0.05$) among leaves, $r^2 = 0.03$, and twigs, $r^2 = 0.01$, between these positions in the canopy (Table 4.3, Figures 4.1 a and b). Differences in microenvironmental factors governing p_i/p_a in the leaves between the two positions in the canopy may explain this phenomenon. Significant correlations ($p < 0.05$) were found among $\delta^{13}C$ values of leaves and supporting twigs from the north, $r^2 = 0.38$, and south, $r^2 = 0.29$, aspects respectively (Figures 4.1 c and d), which suggests that the carbon pool of the supporting twigs may be derived from photosynthates translocated from the leaves

growing on these twigs. The $\delta^{13}C$ values of young leaves did not correlate significantly with any of the plant tissues in the tree canopy which may be explained by different microenvironmental factors governing the p_i/p_a ratios of the leaves at the outer exposed growing tips of branches. Alternatively the carbon isotopic ratio of young tissues may reflect a carbon composition with a large proportion of recently produced photosynthates i.e. sugars and starch.

Table 4.3 Linear correlations between $\delta^{13}C$ of tree tissue types sampled in different positions in a eucalypt canopy. Correlation coefficients are provided for significant correlations at $p < 0.05$. NS indicates $p > 0.05$.

	Leaves north	Twigs north	Leaves south	Twigs south	Young leaves
Leaves north	----	0.62	NS	NS	NS
Twigs north	----	----	NS	NS	NS
Leaves south	----	----	----	0.54	NS
Twigs south	----	----	----	----	NS

4.3.3.2 Relationships between $\delta^{13}C$ of organic constituents extracted from mature leaves.

Correlations among $\delta^{13}C$ of organic constituents and leaf and supporting twig tissues are given in Table 4.4. Among the carbon isotope ratios of leaf organic constituents, a significant correlation ($p < 0.05$) was only found between $\delta^{13}C$ of starch and soluble organics (sugars, amino acids and phenols), $r^2 = 0.43$ (Figure 4.2). This finding suggests that the mobility of the respective carbon pools and fractionation occurring during metabolism may have a substantial influence on the resultant $\delta^{13}C$ values of the organic constituents and that the degree of fractionation may vary with the physiological status of the plant over time.

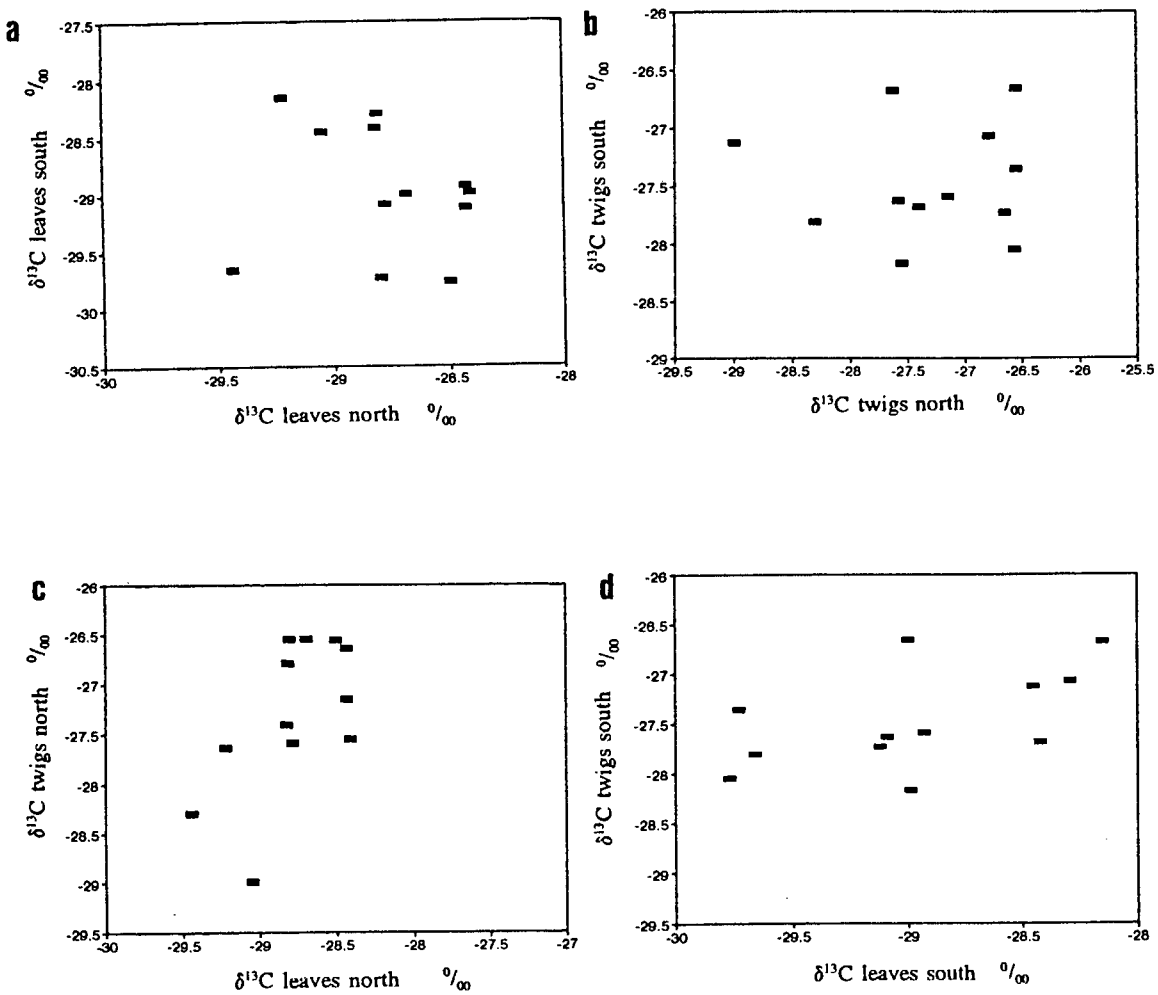


Figure 4.1 The relationships between $\delta^{13}C$ of plant tissues sampled in a eucalypt canopy (a) leaves south and leaves north, $r = 0.17$, $p > 0.05$. (b) twigs south and twigs north, $r = 0.01$, $p > 0.05$ (c) twigs north and leaves north, $r = 0.62$, $p < 0.05$ (d) twigs south and leaves south, $r = 0.54$, $p < 0.05$.

Table 4.4 Linear correlations between $\delta^{13}C$ of organic constituents, whole leaf and supporting twig tissue. Correlation coefficients are provided for significant correlations at $p < 0.05$. NS indicates $p > 0.05$.

	Crude wall fibre	Starch	Soluble organics	Lipids	Leaves north	Twigs north
Crude wall fibre	----	NS	NS	NS	NS	0.59
Starch	----	----	0.66	NS	0.56	0.57
Soluble organics	----	----	----	NS	0.51	NS
Lipids	----	----	----	----	0.69	NS
Leaves north	----	----	----	----	----	0.62

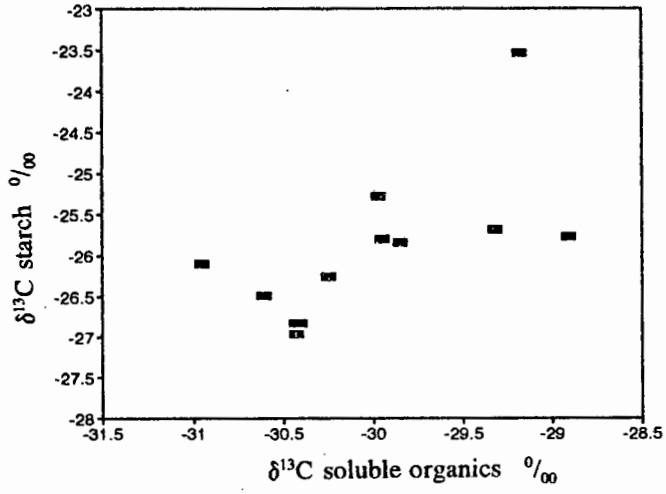


Figure 4.2 The relationship between $\delta^{13}C$ of starch and soluble organics (sugars, amino acids and phenolics) extracted from leaves north, $r = 0.66$, $p < 0.05$.

4.3.3.3 *Relationships among $\delta^{13}C$ of organic constituents and whole leaf and supporting twig tissues*

The $\delta^{13}C$ values of lipids, starch and soluble organics correlated significantly ($p < 0.05$) with $\delta^{13}C$ values of whole leaf tissue from which they were extracted (Figures 4.3 a-c) which suggests that they constitute a large proportion of the carbon pool in mature leaf tissues. The

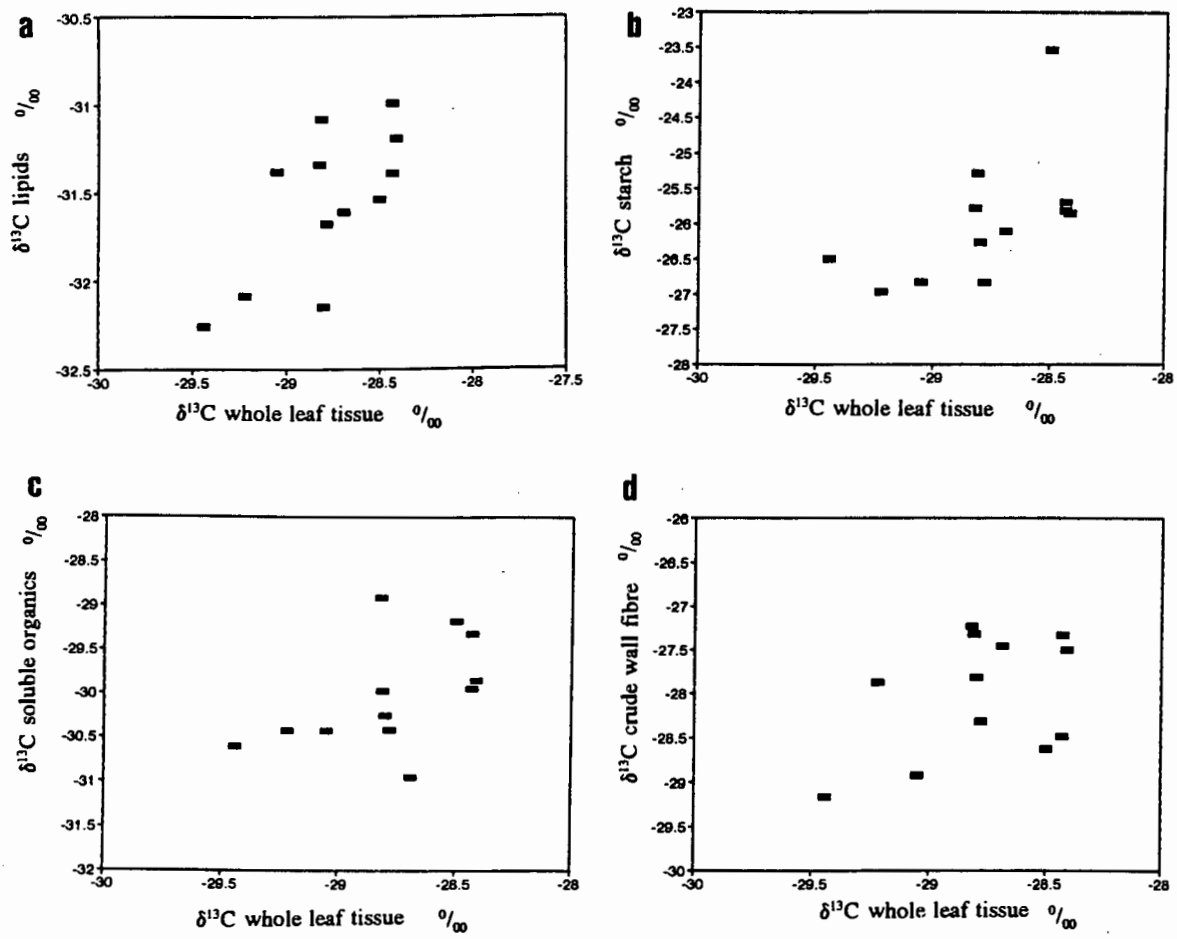


Figure 4.3 The relationships between $\delta^{13}C$ of leaf organic constituents and leaves north (a) lipids and whole leaf tissue, $r = 0.69$, $p < 0.05$ (b) starch and whole leaf tissue, $r = 0.56$ (c) soluble organics (sugars, amino acids and phenolics) and whole leaf tissue, $r = 0.51$, $p < 0.05$ (d) crude wall fibre and whole leaf tissue, $r = 0.42$, $p > 0.05$.

$\delta^{13}C$ of lipids showed the strongest correlation with $\delta^{13}C$ of whole leaf tissue, $r^2 = 0.47$, relative to that of starch and soluble organics, $r^2 = 0.32$ and 0.25 respectively. The $\delta^{13}C$ of crude wall fibre did not correlate significantly ($p > 0.05$) with $\delta^{13}C$ of whole leaf tissue, $r^2 = 0.18$ (Figure 4.3 d). However, $\delta^{13}C$ values of crude wall fibre and starch showed stronger and significant ($p < 0.05$) correlations with $\delta^{13}C$ of supporting twig tissue, $r^2 = 0.35$ and 0.33 respectively (Figures 4.4 a and b), which suggests that the supporting twig tissue

contains a large proportion of structural and storage carbon derived from the leaves.

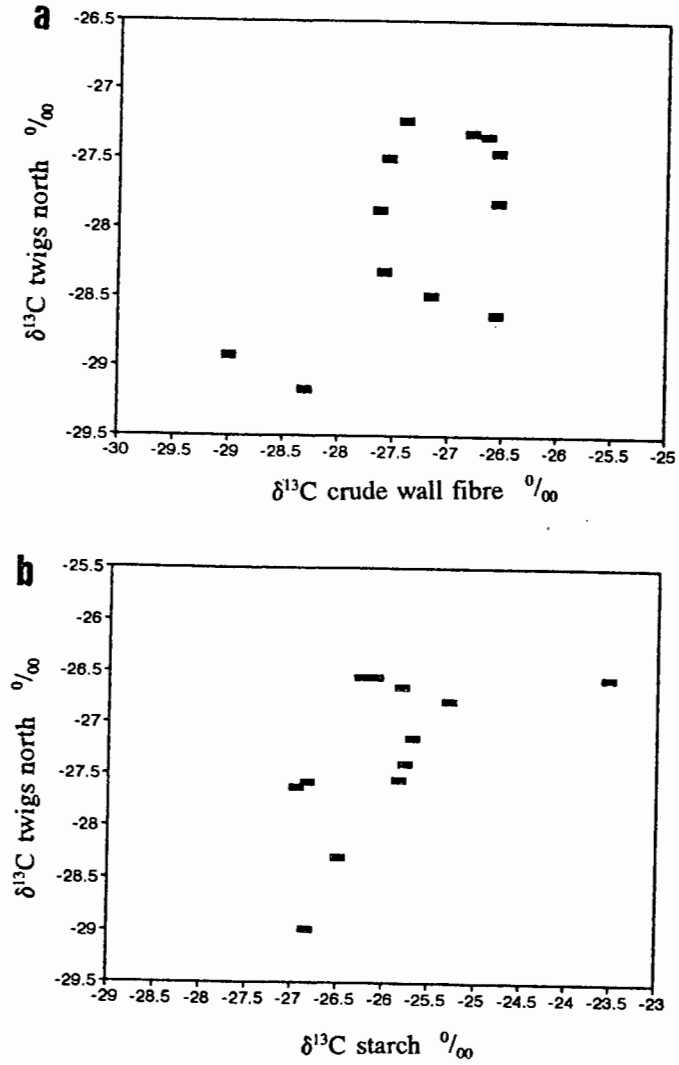


Figure 4.4 The relationships between $\delta^{13}C$ of twigs north and crude wall fibre and starch constituents extracted from leaves north (a) twigs north and crude wall fibre, $r = 0.59$, $p < 0.05$ and (b) twigs north and starch, $r = 0.57$. $p < 0.05$.

4.4 DISCUSSION

Natural variation in carbon isotope ratios between different plant tissues and among various leaf organic constituents is evident in the eucalypt canopies studied. The difference of an average of $1.5‰$ in ^{13}C in twigs relative to adjacent mature leaves conforms to similar findings in *Fagus* (Schleser, 1990; 1992), *Pinus* and *Juniperus* (Leavitt and Long, 1986). The relationship between carbon isotope ratios of mature leaf and adjacent supporting twig

tissue suggests that the carbon pool of twigs is derived directly from the photosynthates translocated from adjacent leaves. The difference between twig and leaf $\delta^{13}C$ cannot be due to any fractionation associated with the translocation of photosynthates such as soluble sugars to the twigs, since this would result in the twig tissue being depleted in ^{13}C (Schleser, 1992). Instead the $\delta^{13}C$ of the twigs are isotopically heavier relative to leaves due to the organic pool being mainly structural and storage carbohydrates. These pools which include crude wall fibre and starch, are isotopically enriched relative to lipids, sugars, amino acids and phenolics. In contrast mature leaf tissues appear to be isotopically lighter mainly due to the influence of the depleted lipid component.

It is suggested that young eucalypt leaf tissue constitutes a larger proportion of sugars and starch relative to lipids and structural carbohydrates, firstly due to the soft texture of the younger leaves and, secondly due to a demand for an energy resource required for further growth of young leaves. A large starch relative to lipid component in the young leaves would result in the isotopic enrichment of the leaf since starch was found to be isotopically enriched by 2 to 5 $^{0}/_{00}$ relative to crude wall fibre, soluble sugars, amino acids, phenols and lipids in this study. Brugnoli et al. (1988) also reports starch to be isotopically enriched by approximately 2.9$^{0}/_{00}$ relative to sugars in poplar and by 1$^{0}/_{00}$ in cotton and bean.

Unlike the finding of differences between carbon isotope ratios between foliage growing on north and south aspects in the canopies of thirteen month old eucalypt clones by Bond and Stock (1990), no significant differences were found between the respective aspects in four year old trees in this study. Similarly no relationships were found between the carbon isotope ratios of foliage between the two aspects. It is possible that differences in microenvironmental factors such as irradiance and humidity could become more pronounced between positions in closed canopies. Consequently the physiological status of leaves may vary substantially between positions in the canopy, resulting in carbon isotope ratios reflecting conditions prevailing at the microsites over time.

The variation in $\delta^{13}C$ between tissues and organic compounds found in this study is suggested to be influenced by varying microenvironmental conditions within the tree canopy and by the relative proportions of differently labelled organic components i.e. crude wall fibre, starch,

lipids and soluble sugars, amino acids and phenols, constituting the mature leaves, supporting twigs and young leaves. This poses a problem in the sampling of plant tissues for $\delta^{13}C$ analyses as indicators of plant physiological traits such as water use efficiency. The most accurate approach would be to choose a tissue that provides a direct link to the physiological processes governing water use efficiency such as leaves, and a carbon component of leaves that would provide the best averaged integral measure of p_i/p_a over the growing season. To meet the first requirement, mature leaves should provide a better estimate of water use efficiency over a longer time scale than young leaves would. To eliminate the influence of the fractionation associated with biochemical metabolism and translocation of organic constituents within leaves, the use of the crude wall fibre component should provide the most accurate estimate of p_i/p_a over the growing season due to its immobility after being formed.

CHAPTER FIVE

WATER USE EFFICIENCY AND $\delta^{13}C$ IN COMMERCIAL CLONES OF *EUCALYPTUS* COMMONLY GROWN IN SOUTH AFRICA

5.1 INTRODUCTION

The $\delta^{13}C$ value of leaf tissues has been proposed to be a potentially useful tool in the selection for water use efficiency (WUE) in crop genotypes of C_3 plants (Farquhar, 1982; Farquhar and Richards, 1984, Farquhar et al., 1988). The theoretical link between WUE and $\delta^{13}C$ is mediated through the independent relationships between WUE and carbon isotope discrimination with stomatal conductance and p_i/p_a (the ratio of leaf intercellular and atmospheric CO_2 concentrations) in the following manner. Instantaneous WUE (the ratio of instantaneous rates of assimilation, A, and transpiration, E, has been described (Farquhar and Richards, 1984) as:

$$A/E = \frac{g_c (p_a - p_i)}{g_w (e_i - e_a)} = \frac{p_a (1 - p_i/p_a)}{1.6v} \qquad (5.1)$$

where g_c and g_w are the conductances to diffusion of CO_2 and water vapour, respectively; e_i and e_a are the intercellular and atmospheric vapor pressures, respectively, and v is the difference between them. The factor 1.6 is the ratio of diffusivities of water vapor and CO_2 in air. WUE over the growth season of a plant has been described (Farquhar et al., 1982) as:

$$WUE_p = \frac{(1 - \phi)\, p_a (1 - p_i/p_a)}{1.6v} \qquad (5.2)$$

where, WUE_p is number of moles of carbon in the plant divided by the number of moles of water transpired during the period of growth, and allowance is made for the proportion of carbon that is fixed for the day but respired by the leaf at night by the factor $(1 - \phi)$. Since carbon isotope discrimination against ^{13}C, has been suggested and empirically shown (Farquhar et al., 1982; Farquhar et al., 1988; von Caemmerer and Evans, 1991, Ehleringer et al., 1992) to increase under high p_i/p_a in the leaf,

$$\delta^{13}C_{pl} = \delta^{13}C_{atm} - a - (b\text{-}a)C_i/C_a \quad (4.1)$$

relatively low WUE and high biomass accumulation should be associated with high p_i/p_a (equation 1 and 2) and tissues depleted in ^{13}C, or having more negative $\delta^{13}C$ signatures. Conversely relatively high WUE and low biomass accumulation should be associated with low p_i/p_a and leaf tissues enriched in ^{13}C, or having less negative $\delta^{13}C$ signatures. The theory holds that the $\delta^{13}C$ signature of the leaf should provide an integral measure of p_i/p_a and hence WUE, over the growing season.

It has been acknowledged that the relationship between p_i/p_a and WUE_p may be complicated by variation in respiratory losses in plants differing in the relative allocation to root and shoot biomass (Farquhar et al., 1988). Also differences in leaf-to-air vapour pressure differences caused by variation in leaf angle and reflectance is not accounted for in the relationship between p_i/p_a and WUE_p (Farquhar and Richards, 1984, Farquhar et al., 1988). Despite the influence of these independent factors on WUE, $\delta^{13}C$ has been shown to be positively correlated with WUE and negatively correlated with dry mass accumulation in tomato, wheat, peanut and potato genotypes (Farquhar and Richards, 1984, Hubick et al., 1986, Martin and Thorstenson, 1988, Vos and Groenwold, 1989). Variation in $\delta^{13}C$ has also been reported to be under strong genetic control in these crop species. Consequently it has been proposed that $\delta^{13}C$ may serve as a useful index for ranking WUE amongst clonal genotypes and species. (Farquhar et al., 1988, Ehleringer et al., 1992). At present, genetic studies of WUE, p_i/p_a and $\delta^{13}C$ are still in their infancy, however Martin et al. (1989) reported that 70% of the variance for discrimination against ^{13}C in a variable tomato population was associated with three discrete DNA sequences within the genome.

The relationship between $\delta^{13}C$ and water use efficiency has not been investigated extensively in trees and it is possible that the effects of respiratory losses and variation in leaf-to-air vapour pressure differences may contribute substantially to the water use efficiency at the whole plant level. Furthermore as was found in four year old trees of *Eucalyptus grandis*, the relationship between $\delta^{13}C$ and WUE of whole tissue samples may be obscurred by the presence of varying concentrations of tissue compounds that may be carrying isotope signatures that are not related directly to p_i/p_a, for example, lipids (chapter 4). As such the aim of this study was to investigate the potential use of $\delta^{13}C$ of whole tissue and extracts of crude wall fibre as a selection tool for WUE in juvenile trees of clonal genotypes commonly grown in South Africa. With this objective in mind, the following questions were addressed:

1. Does $\delta^{13}C$ correlate with p_i/p_a, A/E, season length WUE (WUE_s) and dry mass production?
2. (a) Is there variation in $\delta^{13}C$ values of *Eucalyptus* clones ?
   (b) If so, can this variation in $\delta^{13}C$ be used to predict field performances in terms of productivity and/or WUE ?

5.2. METHODS

5.2.1. $\delta^{13}C$ of whole leaf and crude wall fibre extracts

$\delta^{13}C$ was determined for whole leaf tissue sampled at 10 weeks after planting, for whole leaf tissue upon which gaseous exchange measurements were made at 15½ months and for crude wall fibre extracted from 1g of leaf tissue (chapter 4, see 4.2.1) sampled for the determination of specific leaf areas at 15½ months (chapter 2, see 2.2.8.2). $\delta^{13}C$ values were determined through the process of combustion followed by cryogenic distillation and mass spectrometry (chapter 4, see 4.2.2).

5.2.2. Instantaneous measures of WUE and ratios of p_i/p_a

Instantaneous rates of carbon assimilation (A) and transpiration (E) and p_i/p_a were measured using the LCA_2 infra red gas analyser, air supply unit and narrow Parkinson leaf chamber (The Analytic Development Co. Ltd. Hoddeson, England) in the manner described in chapter 2, section 2.2.9.

5.2.3. Dry mass accumulation and WUE

Dry mass of plants was determined in the manner described in chapter 2, section 2.2.7.2. Water use efficiency with respect to total (WUE_p), shoot (WUE_{sh}) and harvestable stem wood (WUE_h) dry mass, were calculated as the dry mass of the respective plant parts divided by the total litres of water used over the entire growth period as was described in chapter 3, section 3.2.1.

5.2.4. Field performance data

Measurements of stem volume increments of 4 to 5 year old trees of the same clones investigated in this study were recorded in field trials in the eastern Transvaal for the 12 month period extending over the summer drought of 1991/1992. Data were received from Charles Kempthorne, HL&H Tree Breeding Centre, White River.

5.2.5. Statistical analyses

Statistical analyses, data manipulation and graphic display were performed using STATGRAPHICS (Version 5.0, Statistical Graphics Corp., Maryland, USA) and QUATRO

PRO (Version 4.0, Borland International INC., California, USA) software packages.

5.2.5.1 *Relationships among variables*

Simple Linear Regressions were used to determine the relative dependence of the variable in question on another. Regression slopes for the two treatments were tested using Students' t test (Zar, 1984). In the case where the slopes were not significantly different at $p < 0.05$, Simple Linear Regression analyses were performed on the combined data of both W_h and W_l.

5.2.5.2 *Parameters describing clonal and treatment effects*

A Two Way Analysis of Variance model was performed to determine the effects of water availability and genotype on A/E, $\delta^{13}C$ leaf crude wall fibre and $\delta^{13}C$ whole leaf tissue, after which a Least Significant Difference (LSD) Multiple Range test was performed to detect significant differences amongst treatment means and clonal means (treatments not separated) (Zar, 1984).

5.3. RESULTS

5.3.1. $\delta^{13}C$ and p_i/p_a, WUE and dry mass production

The ratios of instantaneous rates of assimilation and transpiration, A/E, $\delta^{13}C$ of whole leaf tissue and leaf crude wall fibre were found to correlate significantly with p_i/p_a (Figures 5.1 a - c). High instantaneous water use efficiencies (A/E) and less negative $\delta^{13}C$ values were found to be associated with low p_i/p_a ratios. Regression analyses showed that the strongest relationship existed between $\delta^{13}C$ of leaf crude wall fibre and p_i/p_a ($r^2 = 0.60$) which implies that $\delta^{13}C$ leaf crude wall fibre may provide a more accurate measure of the discrimination associated with p_i/p_a than $\delta^{13}C$ whole leaf tissue. Similarly $\delta^{13}C$ of crude wall fibre was found to be more strongly positively correlated with A/E than $\delta^{13}C$ whole leaf tissue (Figures 5.2 a and b).

WUE_p was found not to be significantly correlated with instantaneous measures of A/E at the leaf level (Figure 5.3 a) whereas WUE_p was found to be significantly positively correlated with both $\delta^{13}C$ leaf crude wall fibre and whole tissue (Figures 5.4 a and b). However only 15 and 12% of the variability in WUE_p is explained by variation in $\delta^{13}C$ of leaf crude wall fibre and whole leaf tissues, respectively.

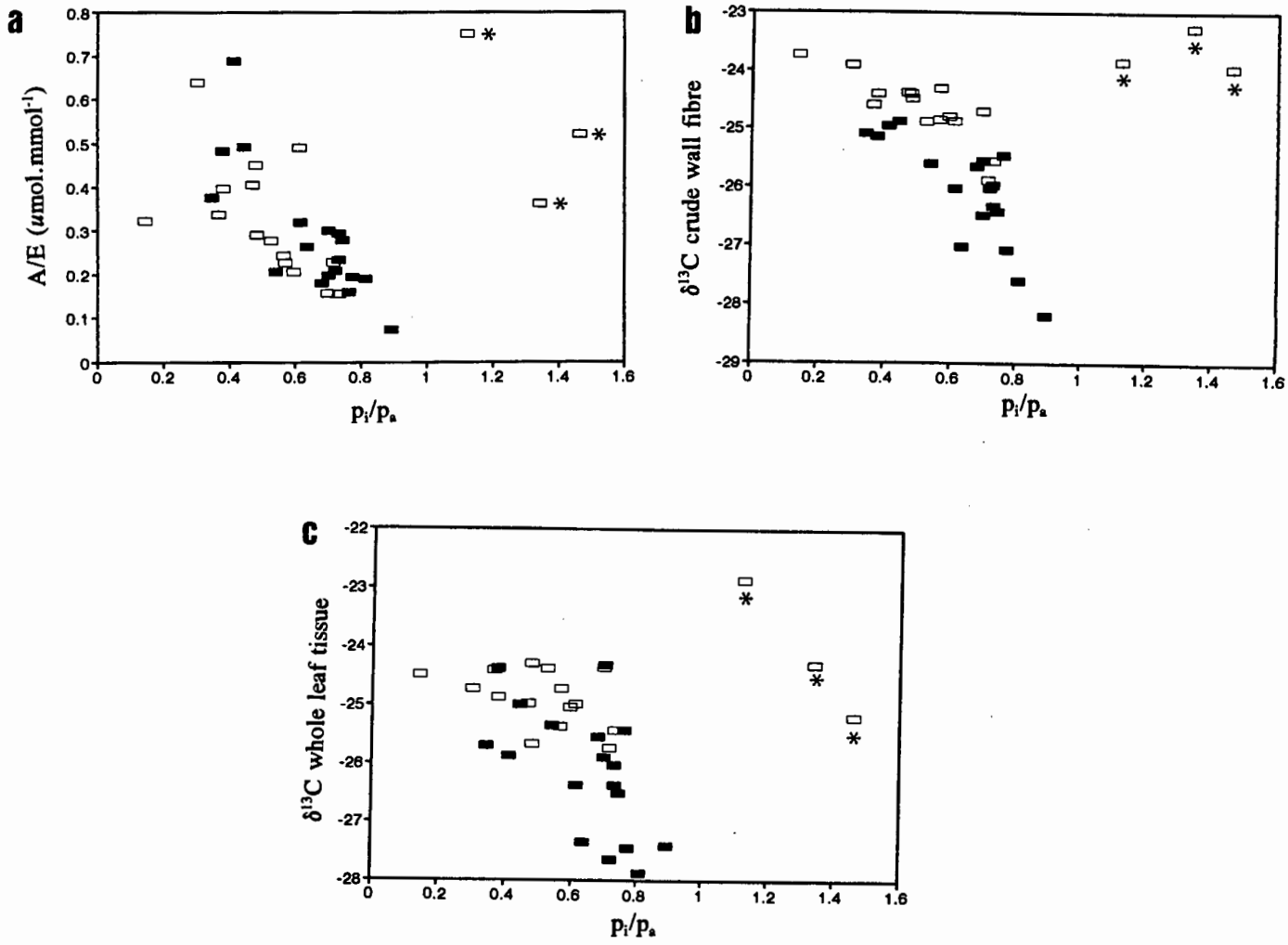


Figure 5.1 (a) Relationship between instantaneous measures of WUE (A/E) and p_i/p_a of 5 leaves upon which gaseous exchange measurements were made. No significant differences between regression slopes of individuals in W_h ■ and W_l □ ($t_{1,29} = 1.22$, $p > 0.05$). A/E = 0.63 - 0.57 p_i/p_a, $r = 0.69$, $n = 33$, $p < 0.05$. (b) Relationship between $\delta^{13}C$ of leaf crude wall fibre (CWF) extracted form *Eucalyptus* leaves at 15½ months and p_i/p_a. No significant differences between regression slopes of individuals of W_h and W_l ($t_{1,29} = 1.62$, $p > 0.05$). $\delta^{13}C$ CWF = -22.63 - 4.82 p_i/p_a, $r = 0.78$, $n = 36$, $p < 0.05$. (c) Relationship between $\delta^{13}C$ of whole leaf tissue at 15½ months and p_i/p_a of 5 leaves upon which gaseous exchange measurements were made. No significant differences between regression slopes of individuals of W_h and W_l ($t_{1,29} = 1.62$, $p > 0.05$). $\delta^{13}C$ whole leaf tissue = -23.39 - 3.73 p_i/p_a, $r = 0.60$, $n = 33$, $p < 0.05$. Points depicted with * were excluded from regression analyses since p_i/p_a ratios were overestimated when stomata were closed.

Stronger significant positive correlations were found between WUE_{sh} and A/E, $\delta^{13}C$ leaf crude wall fibre and $\delta^{13}C$ whole leaf tissue (Figures 5.3 b, 5.4 c and d). Variation in WUE_h was not significantly correlated with variation in instantaneous measures of A/E (Figure 5.3 c) whereas significant positive correlations were found between WUE_h and $\delta^{13}C$ leaf crude wall fibre and whole leaf tissue (Figures 5.4 e and f). Significant negative correlations were found between dry whole plant dry mass and WUE_p, $\delta^{13}C$ leaf crude wall fibre and $\delta^{13}C$ whole leaf tissue (Figures 5.5 a - c). Plant dry mass was strongly linked to $\delta^{13}C$ leaf crude wall fibre ($r^2 = 0.59$).

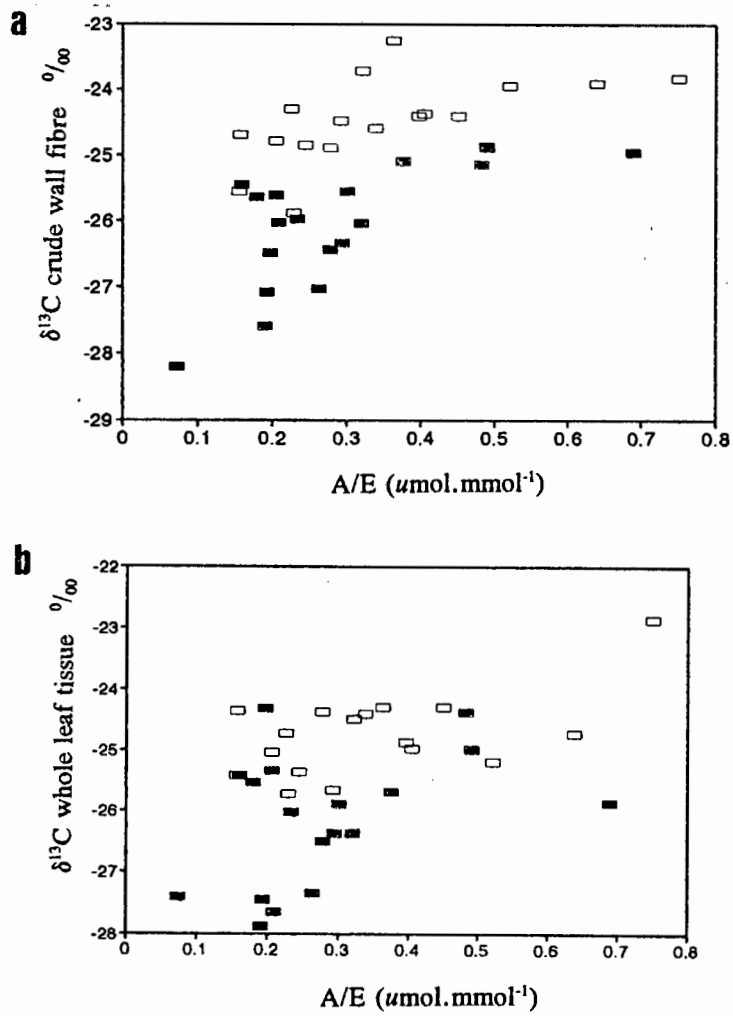


Figure 5.2 (a) Relationship between $\delta^{13}C$ of leaf crude wall fibre (CWF) extracted from *Eucalyptus* leaves at 15½ months and A/E. No significant differences between regression slopes of individuals in W_h ■ and W_l □ ($t_{1,32} = 1.46$, $p > 0.05$). $\delta^{13}C$ CWF = 4.14 A/E - 26.62, $r = 0.58$, $n = 36$, $p < 0.05$. (b) Relationship between $\delta^{13}C$ of whole leaf tissue at 15½ months and A/E. No significant differences between regression slopes of individuals of W_h and W_l ($t_{1,32} = 0.37$, $p > 0.05$). $\delta^{13}C$ whole leaf tissue = 3.39 A/E - 26.5, $r = 0.47$, $p < 0.05$.

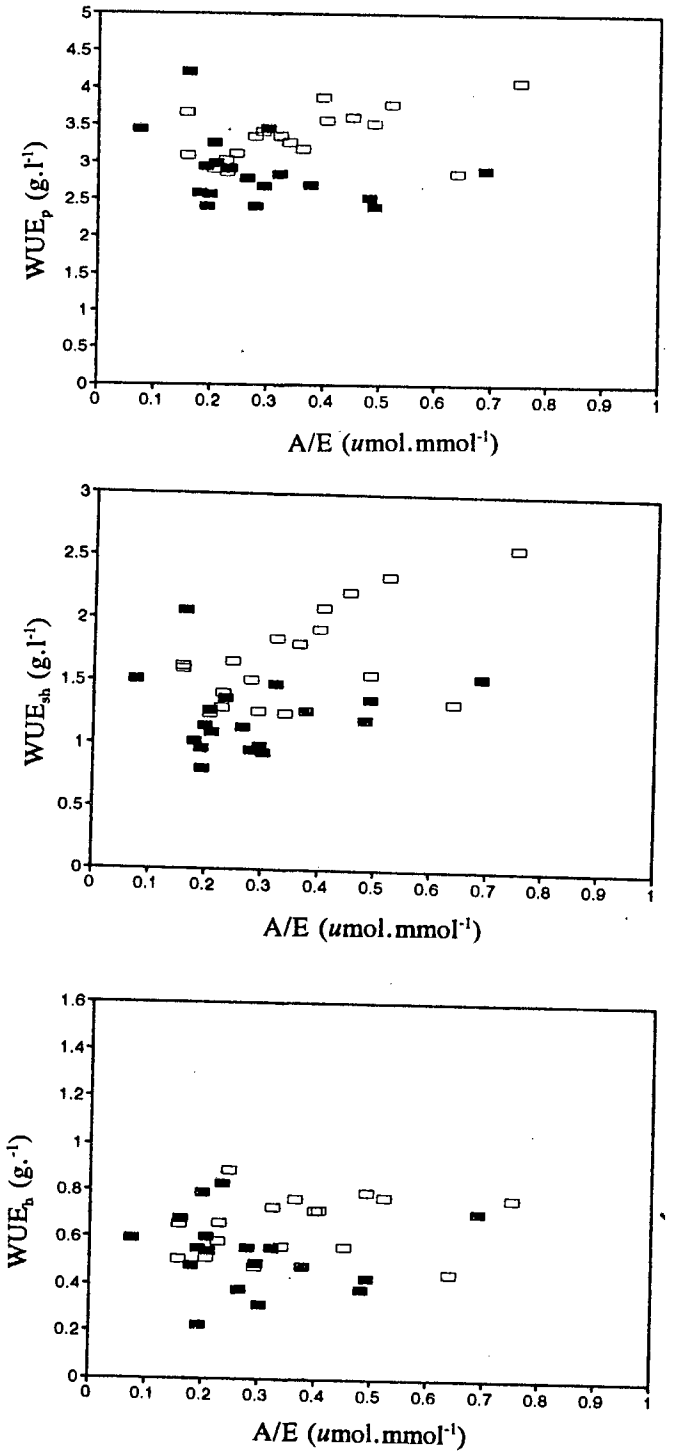


Figure 5.3 (a) Relationship between WUE_p and A/E. Significant differences between regression slopes of individuals in W_h ■ and W_l□ ($t_{1,32} = 2.46$, $p < 0.05$). WUE_p and A/E in W_h and W_l, $r = 0.28$ and 0.35 respectively, $n = 18$, $p > 0.05$. (b) Relationship between WUE_{sh} and A/E. No significant differences between regression slopes between individuals of W_h and W_l ($t_{1,32} = 1.68$, $p > 0.05$). $WUE_{sh} = 1.22$ A/E + 1.07, $r = 0.45$, $n = 36$, $p < 0.05$. (c) Relationship between WUE_h and A/E. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 0.76$, $p > 0.05$) No signficant relationship between WUE_h and A/E, $r = 0.15$, $n = 36$, $p > 0.05$.

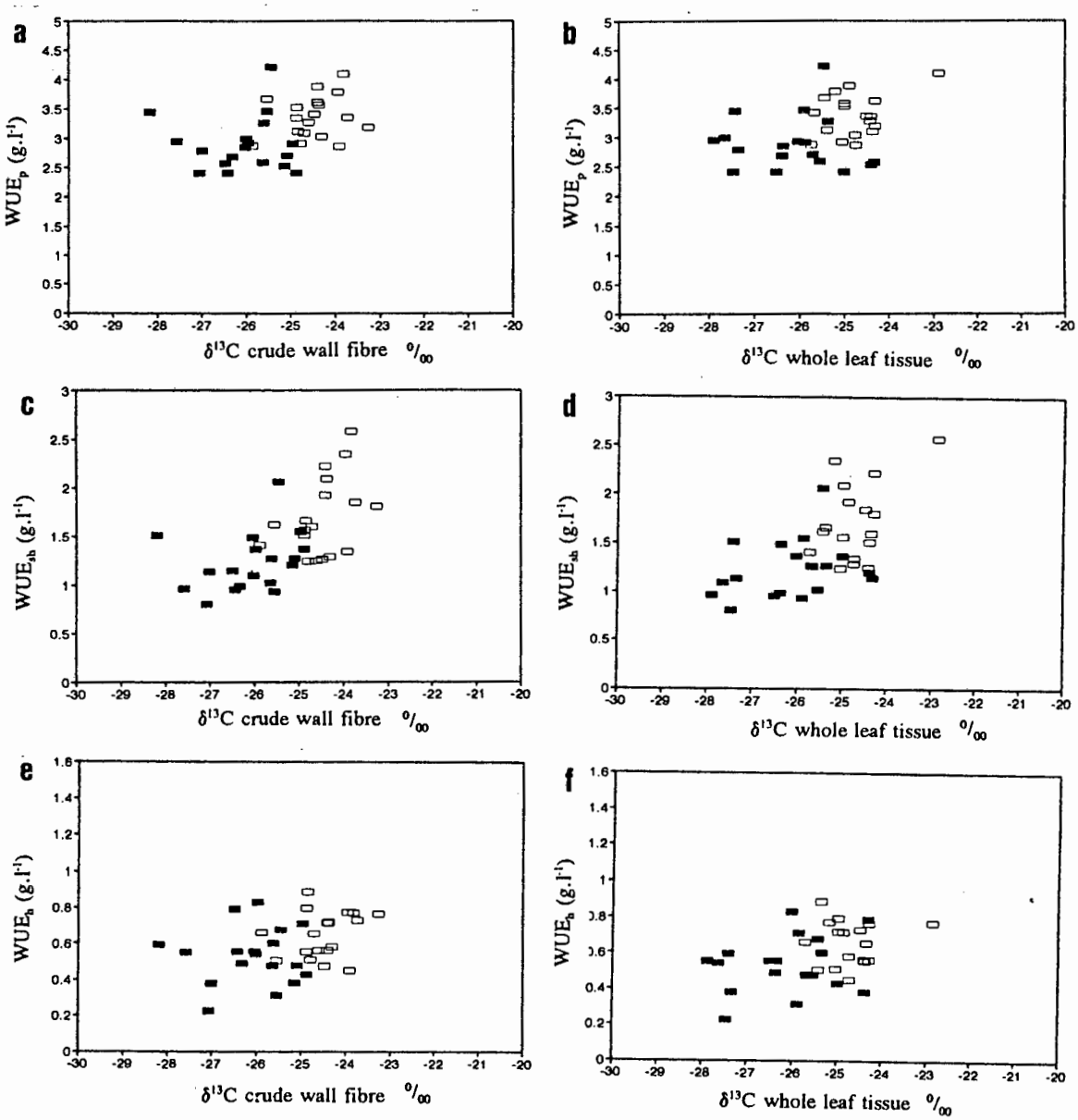


Figure 5.4 (a) Relationship between WUE_p and $\delta^{13}C$ leaf crude wall fibre (CWF) extracted from *Eucalyptus* leaves at 15½ months. No significant differences between regression slopes of individuals in W_h■ and W_l□ ($t_{1,32} = 0.69$, $p > 0.05$). $WUE_p = 0.17\ \delta^{13}C\ CWF + 7.4$, $r = 0.39$, $n = 36$, $p < 0.05$. (b) Relationship between WUE_p and $\delta^{13}C$ whole leaf tissue. No significant differences between regression slopes of individuals of W_h and W_l ($t_{1,32} = 1.05$, $p > 0.05$). $WUE_p = 0.15\ \delta^{13}C$ whole leaf tissue + 7.01, $r = 0.58$, $n = 36$, $p < 0.05$. (c) Relationship between WUE_{sh} and $\delta^{13}C$ CWF. No significant differences between regression slopes of individuals of W_h and W_l ($t_{1,32} = 1.09$, $p > 0.05$). $WUE_{sh} = 0.22$ CWF + 7.11, $r = 0.59$, $n = 36$, $p < 0.05$. (d) Relationship between WUE_{sh} and $\delta^{13}C$ whole leaf tissue. No signficant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 1.55$, $p > 0.05$). shWUE = 0.22 $\delta^{13}C$ whole leaf tissue + 6.96, $r = 0.58$, $n = 36$, $p < 0.05$. (e) Relationship between WUE_h and $\delta^{13}C$ CWF. No significant differences between regression slopes of W_h and W_l ($t_{1,32} = 0.58$, $p > 0.05$). $WUE_h = 0.04$ CWF + 1.77, $r = 0.34$, $n = 36$, $p < 0.05$. (f) Relationship between WUE_h and $\delta^{13}C$ whole leaf tissue. No significant differences between regression slopes of W_h and W_l ($t = 1,32 = 0.06$, $p > 0.05$). $WUE_h = 0.05\ \delta^{13}C$ whole leaf tissue + 1.933, $r = 0.39$, $n = 36$, $p < 0.05$.

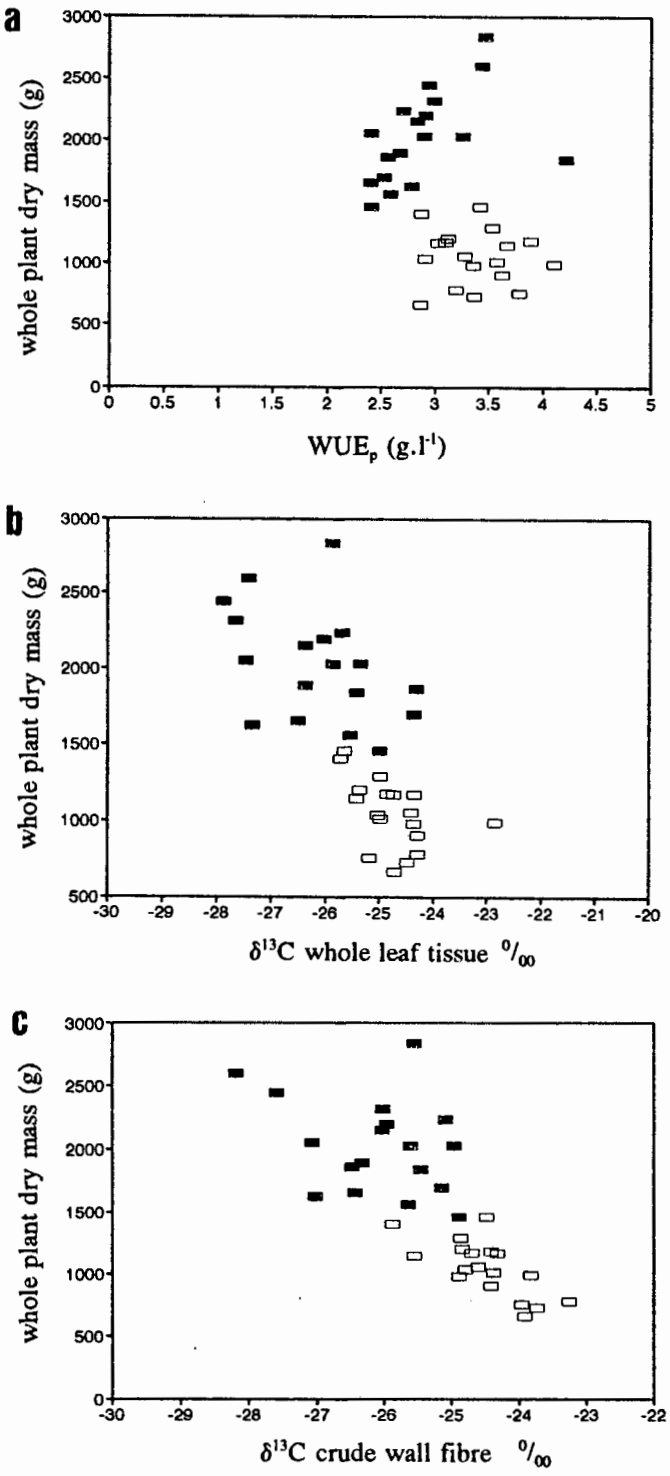


Figure 5.5 (a) Relationship between plant dry biomass and WUE_p. No significant differences between regression slopes of individuals in W_h ■ and W_l □ ($t_{1,32} = 1.39$, $p > 0.05$). Plant dry biomass = 3.51 - 0.00025 WUE_p, $r = 0.30$, $n = 36$, $p < 0.05$. (b) Relationship between plant dry biomass and $\delta^{13}C$ whole leaf tissue at 15½ months. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 0.102$, $p > 0.05$). Plant dry biomass = -7698.16 - 363 $\delta^{13}C$ whole leaf, $r = 0.71$, $n = 36$, $p < 0.05$. (c) Relationship between plant dry weight and $\delta^{13}C$ leaf crude wall fibre (CWF) extracted from *Eucalyptus* leaves at 15½ months. No significant differences between regression slopes of individuals in W_h and W_l ($t_{1,32} = 1.29$, $p > 0.05$). Plant dry biomass = -8492.35 -396.72 δ^{13} CWF, $r = 0.77$, $n = 36$, $p < 0.05$.

5.3.2. Genetic variability in $\delta^{13}C$ in relation to WUE.

Mean clonal instantaneous ratios of assimilation and transpiration (A/E), $\delta^{13}C$ leaf crude wall fibre and $\delta^{13}C$ whole leaf tissue under both high (W_h) and low (W_l) watering treatments are shown in Table 5.1. Significant increases were found in A/E and $\delta^{13}C$ of leaf crude wall fibre and whole leaf tissue under low soil moisture availability (Tables 5.1 and 5.2). No significant genotypic interactions with soil moisture availability were found in A/E, $\delta^{13}C$ leaf crude wall fibre and whole leaf tissue. Significant clonal differences were found in $\delta^{13}C$ leaf crude wall fibre with clones 5 and 6 being significantly more enriched in ^{13}C than clones 1 and 4 (Tables 5.1 and 5.2). This finding coincides with the higher WUE_{sh} in clones 5 and 6 relative to clones 1 and 4 (Figure 5.6).

5.3.3 Predicting clonal WUE from $\delta^{13}C$

$\delta^{13}C$ of whole leaf tissue of 10 week old plants did not show the same pattern of clonal differentiation as in $\delta^{13}C$ whole leaf tissue at 15½ months (Table 5.3). Clones 5 and 6 were found to exhibit higher water use efficiencies than was predicted from $\delta^{13}C$ of 10 week old foliage. The clonal ranking of water use efficiency using $\delta^{13}C$ of leaf crude wall fibre at 15 months was shown to coincide with clonal performances in terms of stem wood growth under drought conditions in the field (Figure 5.7). The more water use efficient clone 6, exhibiting a less negative $\delta^{13}C$ leaf crude wall fibre value was found to be the most productive under water limited conditions. Clone 1,3 and 4 having exhibited lower water use efficiencies and more negative $\delta^{13}C$ leaf crude wall fibre proved to show little or no growth under water limited conditions (Figure 5.7). Clone 5 did not perform according to the isotopic prediction made from $\delta^{13}C$ leaf crude wall fibre.

Table 5.1 Mean clonal A/E, $\delta^{13}C$ whole leaf tissue and $\delta^{13}C$ leaf crude wall fibre measured at 15 months in clones of *Eucalyptus*. Values are means with SE in parenthesis. Standard errors are given in parenthesis. Different letters indicate significant differences at $p < 0.05$. (LSD Multiple Range test after Two way analysis of variance.

Clone	A/E (umol.mmol^{-1})			$\delta^{13}C$ whole leaf			$\delta^{13}C$ leaf crude wall fibre		
	W_h	W_l	Mean (W_h+W_l)	W_h	W_l	Mean (W_h+W_l)	W_h	W_l	Mean (W_h+W_l)
1	0.24 (0.02)	0.32 (0.08)	0.28a (0.11)	-25.61 (0.66)	-25.35 (0.21)	-25.48a (0.78)	-26.29 (0.16)	-25.20 (0.34)	-25.75a (0.73)
2	0.19 (0.06)	0.47 (0.22)	0.24a (0.11)	-26.37 (0.59)	-24.37 (0.06)	-25.37a (1.28)	-26.71 (0.77)	-23.90 (0.42)	-25.30ab (1.82)
3	0.19 (0.01)	0.29 (0.03)	0.24a (0.06)	-27.02 (0.24)	-24.93 (0.37)	-25.98a (1.47)	-26.41 (0.60)	-24.46 (0.08)	-25.44ab (1.26)
4	0.25 (0.03)	0.21 (0.04)	0.23a (0.06)	-26.90 (0.51)	-24.94 (0.31)	-25.92a (1.25)	-26.54 (0.50)	-25.08 (0.24)	-25.81a (1.01)
5	0.45 (0.04)	0.50 (0.07)	0.47a (0.09)	-25.02 (0.38)	-24.66 (0.20)	-24.84a (0.51)	-25.04 (0.08)	-24.23 (0.16)	-24.64b (0.48)
6	0.39 (0.16)	0.52 (0.11)	0.47a (0.22)	-25.88 (0.27)	-24.30 (0.73)	-25.09a (1.21)	-25.48 (0.31)	-24.06 (0.18)	-24.77b (0.87)
mean se	0.29 (0.11)	0.39 (0.13)	----- -----	-26.14 (0.78)	-24.76 (0.40)	----- -----	-26.08 (0.66)	-24.49 (0.54)	----- -----

Table 5.2. Two way analysis of variance listing the effects of clone (n=6) and water treatment (n=2) on A/E, $\delta^{13}C$ whole leaf tissue and $\delta^{13}C$ leaf crude wall fibre. Values are F ratios, *significant differences at $p < 0.05$. NS = not significant.

Leaf traits	Clonal effects	Treatment effects	Interaction effects
A/E	1.515^{NS}	6.087^{*}	0.268^{NS}
$\delta^{13}C$ whole leaf tissue	1.799^{NS}	25.404^{*}	1.604^{NS}
$\delta^{13}C$ leaf crude wall fibre	3.302^{*}	52.487^{*}	1.746^{NS}

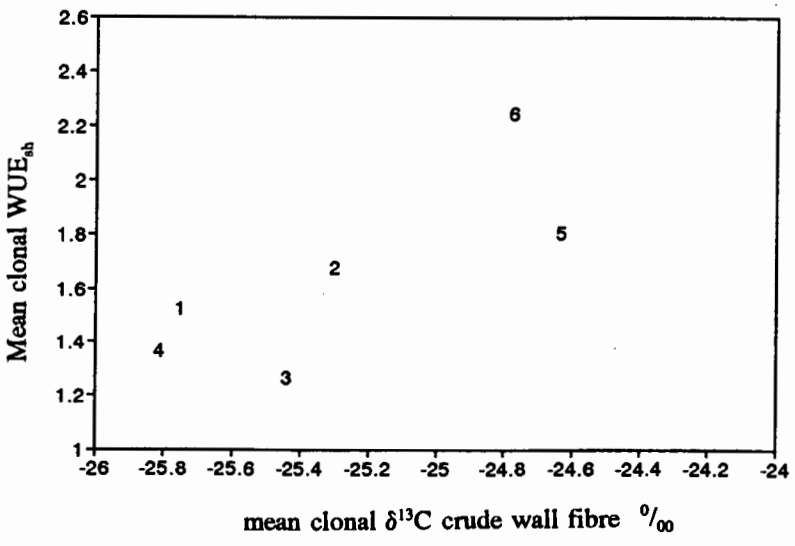


Figure 5.6. Relationship between mean clonal WUE_{sh} and $\delta^{13}C$ leaf crude wall fibre (CWF) extracted from *Eucalyptus* leaves at 15½ months.

Table 5.3. $\delta^{13}C$ of whole leaf tissue sampled at 10 weeks and 15½ months. Clonal means with standard errors (in parenthesis) are given. Ranking from low to high WUE is sequenced from a to f.

Clone	$\delta^{13}C$ at 10 weeks	Predicted ranking in WUE	$\delta^{13}C$ at 15½ months	Ranking in WUE
1	-28.64 (0.47)	a	-25.48 (0.78)	c
2	-27.13 (0.27)	e	-25.37 (0.37)	d
3	-27.62 (0.47)	d	-25.96 (1.47)	a
4	-28.20 (0.36)	b	-25.92 (1.25)	b
5	-27.10 (0.14)	f	-24.84 (0.51)	e
6	-27.90 (0.17)	c	-25.09 (1.21)	f

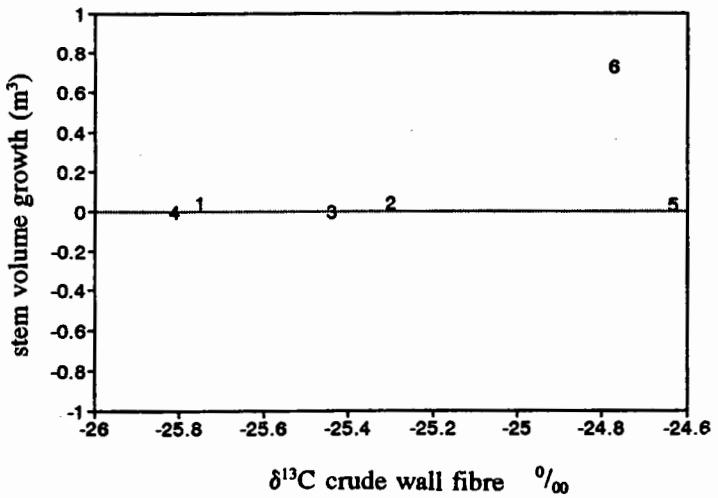


Figure 5.7. Mean clonal stem volume growth (m^3) of 4/5 year old trees under drought conditions and $\delta^{13}C$ leaf crude wall fibre at 15½ months. (Data received from Charles Kempthorne, HL&H Tree Breeding Centre, White River).

5.4. DISCUSSION

This study has shown that $\delta^{13}C$ relates to p_i/p_a in the juvenile leaves of 15½ month old trees of 6 clones of *Eucalyptus* commonly grown in South Africa. Unlike investigations that have been carried out on crop species (Farquhar and Richards, 1984, Hubick et al., 1986, Martin and Thorstenson, 1988), the estimation of p_i/p_a and instantaneous water use efficiency was found to be more strongly related to the $\delta^{13}C$ value of leaf crude wall fibre than $\delta^{13}C$ of whole leaf tissue in eucalypt clones investigated in this study. Similarly the $\delta^{13}C$ values of leaf crude wall fibre was shown to be a more accurate estimate of WUE_p, WUE_{sh} and WUE_h and dry mass production than $\delta^{13}C$ of whole leaf tissue. This finding suggests that $\delta^{13}C$ values of whole leaf tissues of eucalypt clones may be influenced largely by the chemical composition of leaf organic compounds (lipids,sugars and starch) that are carrying isotopic signatures resulting from a secondary fractionation step during metabolism or translocation processes (discussed in chapter 4). Consequently the $\delta^{13}C$ values of whole leaf tissue may not provide an accurate measure of p_i/p_a over the growing season. Crude wall fibre, comprising the more stable and immobile leaf organic pool (lignin and cellulose) has proven to be the more reliable estimate of p_i/p_a and WUE and dry mass production over the growing season as suggested in chapter 4.

Despite the significant relationship between WUE_p and $\delta^{13}C$ in eucalypt clones investigated in this study (Figure 5.4 a), variation in $\delta^{13}C$ of leaf crude wall fibre explained only 15 % of the variation in WUE_p. This contrasts with the findings reported by Martin and Thorstenson (1988) where they showed variation in $\delta^{13}C$ in tomato leaves explained 57 to 65 % of the variability in WUE_p under similar soil moisture availabilities to these used in this study. The improvement in the strength of the relationship between $\delta^{13}C$ of leaf crude wall fibre and WUE_{sh} by approximately 20% ($r^2 = 0.35$, Figure 5.4 c) suggests that the effects of respiratory losses associated with variation in root dry mass (chapter 2) may be obscurring the relationship between whole plant WUE (WUE_p) and $\delta^{13}C$ of leaf crude wall fibre. Also the eucalypts, unlike the tomato plants used by Martin and Thorstenson (1988), allocate a larger proportion of plant mass to root than to shoots. The effect of respiratory losses on $\delta^{13}C$ remains unclear (Farquhar et al., 1988). However variation in respiratory losses associated with large variation in root:shoot ratios will result in a large variation of final WUE derived at a particular p_i/p_a (Farquhar et al., 1988). Although not investigated in this study, a large

proportion of the unexplained variance between $\delta^{13}C$ and WUE_{sh} may be derived from differences in leaf-to-atmospheric vapour pressure differences (VPD) between canopies. These VPD's result from differences in leaf angle and reflectance which are not accounted for in the theoretical link between WUE and p_i/p_a and therefore $\delta^{13}C$ values. This study found however that significant clonal variation in canopy LAI existed (chapter 3) which could imply that leaf density and leaf angle may have had a substantial influence on WUE_p.

Despite the significant correlation between WUE_h and $\delta^{13}C$ leaf crude wall fibre, $\delta^{13}C$ was a poor predictor of WUE_h and this is suggested to be largely due to the fact that harvestable stem dry mass comprises a relatively small proportion of the total plant dry mass in the plants investigated (chapter 2).

Significant differences in mean $\delta^{13}C$ leaf crude wall fibre across treatments coincided with mean clonal season length WUE_{sh} in the eucalypt clones investigated. However accurate predictions of clonal WUE could not be made from $\delta^{13}C$ of leaf tissue as early as ten weeks after planting. However the study showed that clonal growth performances under field conditions at 5 years under water limited conditions coincided with the predictions made from $\delta^{13}C$ values of the leaf crude wall fibre sampled at 15½ months.

The study has shown that $\delta^{13}C$ and water use efficiency are significantly correlated in commercial clones of *Eucalyptus* commonly grown in South Africa. However unlike crop species (Hubick et al., 1986; Martin and Thorstenson, 1988; Vos and Groenwold, 1989), but similar to findings in four year old *Eucalyptus grandis* clones (Olbrich et al., 1993), the relationship appears to be influenced substantially by variation in respiratory losses due to variation in root:shoot ratios and variation in canopy vapour pressure deficits (Hubick et al., 1986, Farquhar et al., 1988, Farquhar et al., 1989). Nevertheless it was shown that the $\delta^{13}C$ of the more stable organic fraction, leaf crude wall fibre provided the best estimate of p_i/p_a, instantaneous and season length water use efficiencies and dry mass production over the growing season. Of greater importance, the relative clonal performance in terms of growth under a low water regime was shown to coincide with the predictions made from leaf crude wall fibre $\delta^{13}C$ values. The major criticisms associated with the technique are that the $\delta^{13}C$ values provide a crude estimate of WUE and do not indicate absolute measures of WUE.

Also $\delta^{13}C$ values may change during plant development and between different sites. However it was shown in this study together with reports made for crop species (Martin and Thorstenson, 1988) that $\delta^{13}C$ is a useful technique for ranking WUE of various clones. Despite the complications associated with the link between $\delta^{13}C$ and WUE mentioned above, within the limited sample size this study showed that the ranking of WUE_{sh} with $\delta^{13}C$ was consistent under different soil moisture availabilities. Therefore this study has indicated that $\delta^{13}C$ is a potentially useful tool in the screening for WUE in clones of *Eucalyptus* commonly grown in South Africa.

CHAPTER SIX

SYNTHESIS

6.1 *Effect of water availability on accumulation of dry mass and water use efficiency*

This study found that growth of commercial clones of *Eucalyptus* is strongly dependent on the availability of soil moisture. Mean total accumulation of dry plant mass and harvestable stem wood was reduced by approximately 52% and 55% respectively, with a corresponding 45% mean reduction in water use under experimental conditions where the available soil moisture was reduced by approximately 50%. Under conditions of 50% lower soil moisture availability, mean increases of 16 and 26% in water use efficiencies with respect to whole plant productivity and harvestable wood were achieved. This plasticity in water use efficiency associated with the change in soil moisture availability indicates the potential for planting eucalypts in areas of marginal rainfall and still obtaining a sustainable increase in yield.

6.2 *Genetic variation in growth rates, dry mass allocation and water use efficiency*

After a growth period of 16 months, no significant differences were found in the relative growth rates and the accumulation of total dry mass at different soil moisture availabilities amongst the 6 commercial clones of *Eucalyptus* investigated in this study. However significant clonal differences were found in dry mass allocation to roots, harvestable wood, side branches and leaves. At both soil moisture levels, two of the *Eucalyptus grandis* clones (clones 3 and 4) were found to invest a significantly higher proportion of dry mass to roots, whereas the *Eucalyptus grandis X nitens* hybrids (clones 5 and 6) were found to invest a larger proportion of dry mass to the leaves. The highest allocation to harvestable wood was found in the *Eucalyptus grandis X camaldulensis* hybrid (clone 1) which was achieved through the lower allocation to side branches and leaves under both soil moisture availabilities. Clonal responses in the allocation of dry mass to harvestable stem under different soil moisture availabilities were significantly different. Two of the *Eucalyptus grandis* clones (2 and 4) responded with an increase in the allocation to harvestable stems, whilst the rest of the clones responded with a decrease. These findings indicate genetic variation in the physiological regulation of carbon partitioning in commercial clones of *Eucalyptus* commonly grown in South Africa. It suggests the potential scope for a breeding programme to increase the carbon allocation to the harvestable stem wood component in eucalypt genotypes to achieve maximum harvestable yield under water limiting conditions.

Although the reduction in total dry plant dry mass and wood produced were found to parallel water use in the 6 eucalypt clones investigated in this study, some clones had higher water use efficiencies than others. The clonal rankings with respect to water use efficiency did not change under the water treatments and the highest water use efficiency was exhibited by the *Eucalyptus grandis X nitens* hybrid (clone 6) across both soil moisture levels.

Due to the clonal variation in biomass allocation patterns, the clonal ranking in water use efficiency was found to be sensitive to the measures of productivity used. For example, the *Eucalyptus grandis X camaldulensis* hybrid (clone 1) had the lowest water use efficiency with respect to total plant dry mass, but the highest water use efficiency with respect to harvestable stem wood because more dry mass was allocated to harvestable stem wood in this clone. Clearly the ranking of water use efficiency based on harvestable stem wood should receive greater priority from the commercial forester. After 16 months of growth, significant clonal variation in the water cost of wood production was found amongst the commercial genotypes of *Eucalyptus* studied. The *Eucalyptus grandis X camaldulensis* (1) and *Eucalyptus grandis X nitens* (6) clones used approximately 1083 and 985 litres less water than the *Eucalyptus grandis* (4) clone to produce 1 kg of harvestable stem wood (Table 1). Further extrapolation to clonal water use per hectare after 16 months of growth show that the water cost of a 1 hectare plantation of *Eucalyptus grandis* X *nitens* (clone 6) would be approximatley 30% lower than a 1 hectare plantation of *Eucalyptus grandis* (clone 4) (Table 6.1). It is possible that clonal differences in the water cost of wood production may be magnified in older trees as Olbrich et al. (1993) showed that the cost of 1 m^3 wood production could differ by approximately 46 m^3 water amongst 4 year old clones of *Eucalyptus grandis*. Therefore there is increasing evidence that the selection for, and planting of more water use efficient clones in existing and new plantations would have a significant influence on the hydrological costs associated with the planting of *Eucalyptus* in South Africa.

Table 6.1 The water cost of wood production and a 1 hectare plantation after a 16 month growth period of commercial clones of *Eucalyptus* commonly grown in South Africa. Clonal means and standard errors (in parenthesis) include data for high and low water treatments.

Clone identification	WUE of harvestable wood production ($g.l^{-1}$)	Extrapolation to litres water per kilogram wood production	Tree density per hectare at 1 X 2 m espacement*	Water use $m^3.ha^{-1}$
grandis X camaldulensis (1)	0.754 (0.12)	1326	5000	2718
grandis (2)	0.641 (0.10)	1560	5000	2410
grandis (3)	0.533 (0.04)	1876	5000	2747
grandis (4)	0.415 (0.13)	2410	5000	2600
grandis X nitens (5)	0.505 (0.12)	1980	5000	2003
grandis X nitens (6)	0.703 (0.08)	1422	5000	1892

* Optimal espacement in *Eucalyptus grandis* plantations (Cristie and Button, 1991)

** Water use per hectare = $1/WUE_p$ ($kg.l^{-1}$) * dry plant mass (kg) * tree density per hectare

6.3 *Physiological processes controlling growth and water use efficiency*

For the 6 eucalypt clones studied, higher relative growth rates were achieved through higher physiological activity (photosynthetic and respiratory activity) per unit leaf area as indicated by the positive correlations between relative growth rate and net assimilation ratio (Poorter, 1989) under both water regimes. Growth was found to correlate positively with the high allocation of dry mass to roots resulting in high root:shoot, root:leaf area ratios, and large canopy leaf areas with low specific leaf areas.

Increases in total biomass accumulation did not correlate with instantaneous rates of photosynthesis per unit leaf area. It is suggested that factors such as the small leaf sample size measured in the canopy, and the influence of environmental factors such as leaf to air vapour pressure deficits (VPD), on stomatal conductance and rates of photosynthesis at the time of the single measurement taken in the field, could obscure the relationship. However,

similar to work undertaken on *Eucalyptus globulus* in Portugal (Pereira et al., 1993) increases in growth were achieved through increases in canopy leaf areas under sufficient water supply. Similar to nutrition experiments on *Eucalyptus grandis* in Australia (Sands et. al, 1992) growth responses under low water availability were achieved through increases in foliar nitrogen concentrations and lower specific leaf area with the reduction in canopy leaf area.

The allocation to harvestable stem wood in clones was found to be a consequence of the trade off with branch and leaf biomass under sufficient water supply. Under conditions of lower soil moisture availability stem dry mass production was more dependent on plant traits influencing the net productivity rather than allocation (i.e. increase in roots and low specific leaf areas). Nutritional studies carried out on seedlings of *Eucalyptus grandis* in Australia have shown that enhanced rates of nitrogen uptake result in increased allocation of carbon to foliage and stems at the expense of roots (Cromer and Jarvis, 1990). This coincides with the predictions made from the biomass allocation model developed by Thornley and Reynolds (1982), where a high C:N ratio in the plant leads to increased carbon export to the roots but at a low C:N ratio leaf growth is promoted. Even though the effects of nitrogen on allocation patterns were not included in this experiment, genetic variation in allocation patterns was expressed under the given soil nitrogen concentrations existing in the pots throughout the experimental growth period. However the physiological explanation for the genetic variation in dry mass allocation patterns to functional organs such as roots, branches, stems and leaves remains unknown.

Clonal variation in water use efficiency at the whole plant level was shown to be correlated with clonal variation in canopy density (leaf area index) and foliar nitrogen concentrations per unit area (Chapter 3). Clones with dense canopies and high foliar nitrogen concentrations per unit area exhibited enhanced water use efficiencies. This pattern is probably linked to the maintenance of higher relative humidities within the canopy boundary layer of dense canopies and higher photosynthetic carbon gain per unit water transpired in leaves with high foliar nitrogen concentrations per unit area. The water cost of harvestable stem wood production decreased with increases in foliar nitrogen concentrations per unit area and increases in allocation to above ground plant parts relative to roots. Based on the principle

effects of C:N ratios on the allocation patterns to above and below ground dry mass in *Eucalyptus grandis* (Cromer and Jarvis, 1990), the correlation between a low water cost of wood production with high leaf nitrogen concentrations per unit leaf area found in this study suggests that the water cost per unit wood production would be reduced with a decrease in C:N ratios in the plant for the following reason. Under a low C:N ratio, it is expected that leaf growth should be promoted and a higher allocation of biomass should be allocated to above ground parts with high nitrogen concentrations in the leaves (Cromer and Jarvis, 1990). This should promote a high rate of carbon assimilation relative to the unit water transpired. This implies that fertilization with nitrogen should result in higher water use efficiencies in *Eucalyptus*.

6.4 *Selection for productivity and water use efficiency*

The negative correlation between whole plant dry mass accumulation and water use efficiency (chapter 5) suggests conflicting requirements when selecting for productivity or water use efficiency in commercial clones of *Eucalyptus*. However this study has indicated that significant clonal variation in biomass allocation patterns to harvestable stem wood exists. Therefore plant traits associated with increases in the harvestable stem wood component and decreases in the water cost of wood production would serve as a useful basis in selecting for sustainable yield and improved water use efficiency.

Nitrogen per unit leaf area and stable carbon isotope ratios of the crude wall fibre were correlated significantly with clonal water use efficiency ($r = 0.39$ for both variables, chapters 3 and 5). High water use efficiency was achieved through high nitrogen per unit leaf area due to high rates of carbon assimilation per unit water loss at lower p_i/p_a (Figure 6.1). Water use efficiency in terms of harvestable stem wood production was shown to be correlated more strongly with nitrogen per unit area ($r^2 = 0.32$, chapter 3) than with the $\delta^{13}C$ of crude wall fibre ($r^2 = 0.11$, chapter 5). However the use of foliar nitrogen concentrations as an index is complicated by possible changes in the C:N ratios under changing soil nutrient availabilities.

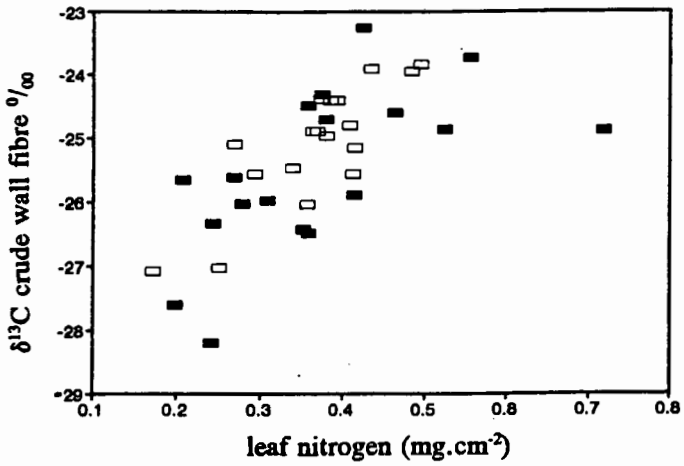


Figure 6.1 The relationship between $\delta^{13}C$ leaf crude wall fibre and foliar nitrogen per unit leaf area for 16 month clones of *Eucalyptus*. Regression analyses on data of both watering treatments, $r = 0.66$, $p < 0.05$.

Although $\delta^{13}C$ values of leaf crude wall fibre did not correlate significantly with the water use efficiency of harvestable stem wood (Chapter 5), significant relationships between $\delta^{13}C$ of crude wall fibre and water use efficiency and dry mass accumulation at the whole plant level were found in accordance to the predictions made by Farquhar et al. (1982). Unlike crop annuals investigated (Farquhar and Richards, 1984, Hubick et al., 1986, Martin and Thorstenson, 1988) the relationship between $\delta^{13}C$ and water use efficiency found in this study and by Olbrich et al. (1993) appears to be more complicated in trees. The effects of factors such as respiratory losses associated with large root:shoot ratios and varying VPD due to varying leaf canopy densities may account for the large proportion of unexplained variance in the relationship between $\delta^{13}C$ values of leaf crude wall fibre and water use efficiency (Farquhar et al., 1989, Hubick, 1990). Nevertheless, the clonal variation in $\delta^{13}C$ of leaf crude wall fibre was shown to reflect clonal variation in water use efficiencies at 16 months. Despite the small sample size, $\delta^{13}C$ of leaf crude wall fibre of 16 month old seedlings was shown to be a possible tool for the ranking of clonal performances in 5 year old trees in field trials experiencing water shortages during the 1991/1992 drought in the Eastern Transvaal.

This study has revealed that large clonal variation exists in harvestable dry mass accumulation and water use efficiency in juvenile *Eucalyptus* grown in South Africa. The ranking of clonal water use efficiency at 16 months provided an indication of clonal growth performances in mature trees under conditions of water shortage. This suggests scope for a breeding programme for increased harvestable wood production and water use efficiency under limited water availability in commercial clones of *Eucalyptus*. At this stage of research the combination of foliar nitrogen per unit area and the $\delta^{13}C$ of leaf crude wall fibre could offer a possible means for ranking clonal water use efficiencies and allocation to leaf and stem tissues. However further work needs to be carried out to support this suggestion.

6.5 *Future recommendations*

The results derived from this study suggest two possibilities.

Firstly, experiments investigating the influence of nitrogen nutrition on the photosassimilate partitioning and the consequent effects on the water cost per unit wood production, should be carried out on commonly grown commercial clones of *Eucalyptus* in South Africa. Information derived from such experiments would indicate whether clonal responses to increased nitrogen supply are significantly different and whether increased nitrogen supply results in the increased allocation to leaves and stems relative to roots and increased water use efficiency. Futhermore the ranking of clonal harvestable biomass production and water use efficiency based upon clonal variation in foliar nitrogen per unit area should be tested. If foliar nitrogen per unit area proves to be a reliable indicator of the cost of wood production and water use efficiency, it would provide a relatively cheap and easy technique to screen for these traits.

Secondly, the relationship between $\delta^{13}C$ and water use efficiency in commercial clones of *Eucalyptus* commonly grown in South Africa can be improved if estimates of respiratory losses associated with variation in root:shoot ratios, as well as the non stomatal water loss can be made (Farquhar et al. 1989). It has been suggested that the respiratory costs of growth could be estimated using the glucose equivalents of the elemental composition (C, N, H, O) of plant material (McDermit and Loomis, 1981). Furthermore the use of oxygen and deuterium isotopes in plant tissues have been suggested to provide integral measures of

vapour pressure deficits weighted by stomatal conductance over the growing season (Farquhar et al., 1988). A study incorporating the combination of these techniques to estimate respiratory losses associated with high root:shoot ratios and differences in canopy vapour pressure deficits caused by variation in leaf angle and density, may be useful in refining the relationship between $\delta^{13}C$ and water use efficiency in commerical clones of *Eucalyptus*.

REFERENCES

Barnes, D.K. (1983) Managing root systems for efficient water use: Breeding plants for efficient water use. In: Limitations to efficient water use in crop production. (Eds. H.M. Taylor, W.R. Jordan and T.R. Sinclair) pp 127-136. American Science of Agronomy, Inc.

Bender, M.M. (1968) Mass spectrometric studies in carbon-13 variations in corn and other grasses. *Radiocarbon, 10:468-472.*

Benner, R, Fogel, M.L. Sprague, E.K. and Hodsons, R.E. (1977) Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature, 329:708-710.*

Blackman, G.E. and Wilson, G.L. (1951) Physiological and ecological studies in the analysis of plant environment. *Annals of Botany, 15(59):373-408.*

Bloom, A.J., Chapin III, F.S. and Mooney, H.A. (1985) Resource limitation in plants - An economic analogy. *Annual Review of Ecological Systematics, 16:363-392.*

Blum, A. (1974a) Genotypic responses in sorghum to drought stress: I. Response to soil moisture stress. *Crop Science, 14:361-364.*

Blum, A. (1974b) Genotypic responses in sorghum to drought stress. II. Leaf tissue water relations. *Crop Science, 14:691-692.*

Boden, D.I. (1991) The relationship between soil water status, rainfall and the growth of *Eucalyptus grandis. South African Journal of Forestry, 156:49-55.*

Bond, W.J. and Stock, W.D. (1990) Preliminary assessment of the grading of *Eucalyptus* clones using carbon isotope discrimination. *South African Journal of Forestry, 154:51-55.*

Bosch, J.M. and von Gadow, K. (1990) Regulating afforestation for water conservation in South Africa. *South African Journal of Forestry, 153:41-54.*

Bosch, J.M. and Smith, R.E. (1989) The effect of afforestation of indigenous scrub forest with *Eucalyptus* on streamflow from a small catchment in the Transvaal, South Africa. *South African Journal of Forestry, 150:7-17.*

Brugnoli, E., Hubick, K.T., von Caemmerer, S., Wong, W.C. and Farquhar, G.D. (1988) Correlation between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide. *Plant Physiology, 88:1418-1424.*

Burton, G.W., Hanna, W.W., Johnson, J.C. Jr., Leuck, D.B., Monson, W.C., Powell, J.B., Wells, H.D. and Widstrom, N.W. (1977) Pleiotropic effects of the *tr* trichomeless gene in pearl millet on transpiration, forage quality, and pest resistance. *Crop Science, 17:613-616.*

Ceulemans, R.J. (1989) Genetic variation in functional and structural productivity components in *Populus*. In: Causes and consequences in growth rate and productivity of higher plants. (Eds. H. Lambers, M.L. Cambridge, H. Konings, T.L. Pons) pp71-85. SPB Academic Publishing. The Hague.

Christie, S. and Button, S. (1991) The biomass production of *Eucalyptus grandis* planted at various close espacements. In: Intensive Forestry: The role of Eucalyptus. 2:891-900. IUFRO symposium, Durban, South Africa.

Cole, D.F., Dobrenz, A.K., Massengale, M.A. and Neal Wright, L. (1970) Water requirement and its association with growth components and protein content of alfalfa (*Medicago sativa* L.). *Crop Science, 10:237-240.*

Cowan, I.R. and Farquhar, G.D. (1977) Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology, 31:471-505.*

Cromer, R.N. and Jarvis, P.G. (1990) Growth and biomass partitioning in *Eucalyptus grandis* seedlings in response to nitrogen supply. *Australian Journal of Plant Physiology, 17(5): 503-516.*

De Niro, M.J. and Epstein, S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science,197:261-263.*

Dye, P.J. and Olbrich, B.W. (1993) Estimating transpiration from 6-year-old *Eucalyptus grandis* trees: development of a canopy conductance model and comparison with independent sap flux measurements. *Plant, Cell and Environment, 16:45-53.*

Ehleringer, J.R., Field, C.B., Zhi-fang Lin and Chun-yen Kuo (1986) Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia, 70:520-526.*

Ehleringer, J.R., Phillips, S.L. and Comstock, J.P. (1992) Seasonal variation in the carbon isotopic composition of desert plants. *Functional Ecology, 6:396-404.*

Evans, J.R. (1991) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia, 78:9-19.*

Farquhar, G.D., O'Leary, M.H. and Berry, J.A. (1982) On the Relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology, 9:121-137.*

Farquhar, G.D. and Richards, R.A. (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology, 11:539-552.*

Farquhar, G.D., Hubick, K.T., Condon, A.G. and Richards, R.A. (1988) Carbon isotope fractionation and plant water-use efficiency. In: Stable Isotopes in Ecological Research. (Eds. P.W. Rundel, J.R. Ehleringer and K.A. Nagy) Ecological Studies No.68. Springer-Verlag, New York. pp21-40.

Farquhar, G.D., Ehleringer, J.R. and Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology, 40:503-537.*

Farrar, J.F. (1989) The carbon balance of fast-growing and slow-growing species. In: Causes and Consequences in Growth Rate and Productivity of Higher Plants. (Eds. H. Lambers) pp 241-267.

Francey, R.J., Gifford, R.M., Sharkey, T.D. and Weir, B. (1985) Physiological influences on carbon isotope discrimination in huon pine (*Lagarostrobos franklinii*). *Oecologia, 66:211-218.*

Gebauer, G. and Schulze, E.-D. (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. *Oecologia, 87:198-207.*

Gifford, R.M. and Thorne, J.H. (1984) Crop productivity and photoassimilate partitioning. *Science, 225:801-808.*

Grime, J.P. and Hunt, R. (1975) Relative growth-rate:its range and adaptive significance in local flora. *Journal of Ecology, 63:393-422.*

Hubick, K.T., Farquhar, G.D. and Shorter, R. (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Australian Journal of Plant Physiology, 13:803-816.*

Hubick, K. (1990) Effects of nitrogen source and water limitation on growth, transpiration efficiency and carbon isotope discrimination in peanut cultivars. *Australian Journal of Plant Physiology, 17(4): 413-430.*

Hunt, O.J. (1962) Water requirement of selected genotypes of *Elymus junceus* Fisch. and *Agropyron intermedium* (Host) Beauv. and their parent-progeny relationships. *Crop Science, 2:97-99.*

Hunt, R. (1978) Plant growth analysis. Studies in Biology No 96. Camelot Press Ltd. Great Britain. 67p.

Hunt, R. and Lloyd, P.S. (1987) Growth and partitioning. *New Phytologist, 106 (suppl.):235-249.*

Jarvis, P.G. (1976) The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society of London., 273:593-610.*

Jewitt, G.P.W. and Schulze, R.E. (1991) A decision support system for assessing hydrological impacts of afforestation. In: Proceedings of the Fifth South African National Hydrological Symposium. pp5B.1.1-5B.1.9. Stellenbosch.

Keeling, C.D., Mook, W.G. and Tans, P.P. (1979) Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. *Nature, 277:121.*

Keyworth, P. (1993) Eucalyptus Timber Markets - demand remains keen. *Eucalypt News, No 3:1.*

Konings, H. (1989) Physiological and morphological differences between plants with a high NAR or a high LAR as related to environmental conditions. In: Causes and Consequences in Growth Rate and Productivity of Higher Plants. (Eds. H. Lambers, M.L. Cambridge, H. Konings, T.L. Pons) pp101-123. SPB Academic Publishing. The Hague.

Lambers, H., Freijsen, N., Poorter, H., Hirose, T. and van der Werf, A. (1989) Analysis of growth based on net assimilation rate and nitrogen productivity. Their physiological background. In: Causes and Consequences in Growth Rate and Productivity of Higher Plants. (Eds. H. Lambers, M.L. Cambridge, H. Konings, T.L. Pons) pp1-17. SPB Academic Publishing. The Hague.

Landsberg, L.L. (1986) Physiological Ecology of Forest Production. Academic Press, London. 198p

Leavitt, S.W. and Long, A. (1986) Stable-carbon isotope variability in tree foliage and wood. *Ecology, 67:1002-1010.*

MacVicar, C.N., Loxton, R.F., Lambrechts, J.J.N., Le Roux, J., De Villiers, J.M., Verster, E., Merryweather, F.R. and van Rooyen, T.H. (1977) Soil Classification. A Binomial System for South Africa. Science Bulletin, 390. The Department of Agricultural Technical Services. Republic of South Africa.

Malan, F.S. (1991) Variation, association and inheritance of juvenile wood properties of *Eucalyptus grandis* Hill ex Maiden with special reference to the effect of rate of growth. *South African Journal of Forestry, 157:16-23.*

Martin, B. and Thorstenson, Y.R. (1988) Stable carbon isotope composition ($\delta^{13}C$), water use efficiency, and biomass productivity of *Lycopersicon esculentum, Lycopersicon pennellii,* and the F_1 hybrid. *Plant Phyisology, 88:213-217.*

Martin, B., Nienhuis, J., King, G. and Schaefer, A. (1989) Restriction fragment length polymorphisms associated with water use efficiency in tomato. *Science, 243:1725-1728.*

McDermitt, D.K. and Loomis, R.S. (1981) Elemental composition of biomass and its relation to energy content, growth efficiency, and growth yield. *Annals of Botany, 48:275-290.*

Medina, E. and Minchin, P. (1980) Stratification of $\delta^{13}C$ values in Amazonian forests. *Oecologia, 45:377-378.*

Medina, E., Montes, G., Cuevas, E. and Rokcsandic, Z. (1986) Profiles of CO_2 concentration and $\delta^{13}C$ values in tropical rain forests of the Upper Rio Negro basin, Venzuela. *Journal of Tropical Ecology 2:207-217.*

Medina, E., Sternberg, L. and Cuevas, E. (1991) Vertical stratification of $\delta^{13}C$ values in closed natural and plantation forests in the Luquillo mountains, Puerto Rico. *Oecologia, 87:369-372.*

Mooney, H.A. (1972) The carbon balance of plants. *Annual Review of Ecological Systematics, 3:315-346.*

Mooney, H.A., Ferrar, P.J. and Slayter, R.O. (1978) Photosynthetic capacity and carbon allocation patterns in diverse growth forms of *Eucalyptus. Oecologia, 36:103-111.*

Mooney, H.A. and Winner, W.E. (1991) Partitioning response of plants to stress. In: Responses of Plants to multiple stresses. (Eds. H.A. Mooney, W.E. Winner, E.J. Pell, and E. Chu). pp129-141. Academic Press, Sandiago.

Nier, A.O. and Gulbransen, E.A. (1939) Variations in the relative abundance of the carbon isotopes. *Journal of the American Chemical Society, 61:697-698.*

Olbrich, B.W. (1991) The verification of the heat pulse velocity technique for estimating sap flow in *Eucalyptus grandis. Canadian Journal of Forest Research 21:836-841.*

Olbrich, B.W., Le Roux, D., Poulter, A.G., Bond, W.J. and Stock. W.D. (1993) Variation in water use efficiency and $\delta^{13}C$ levels in *Eucalyptus grandis* clones. *Journal of Hydrology (in press).*

O'Leary, M.H. (1981) Carbon isotopes in photosynthesis. *BioScience, 38(5):328-336.*

Park, R. and Epstein, S. (1961) Metabolic fractionation of C^{13} & $C^{12}C$ in plants. *Plant Physiology, 36:133-138.*

Pearcy, R.W. and Pfitsch, W.A. (1991) Influence of sunflecks on the $\delta^{13}C$ of *Adenocaulon bicolor* plants occurring in contrasting forest understorey microsites. *Oecologia, 86:457-462.*

Pereira, J.S., Chaves, M.M., Fonseca, F., Araújo, M.C. and Torres, F. (1993) Photosynthetic capacity of leaves of *Eucalyptus globulus* (Labill.) growing in the field with different nutrient and water supplies. *Tree Physiology (in press)*

Poorter, H.P. (1989) Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In: Causes and Consequences in Growth Rate and Productivity of Higher Plants. (Eds. H. Lambers, M.L. Campbridge, H. Konings, T.L.) pp 45-68. SPB Academic Publishing, The Hague.

Read, J.J., Johnson, D.A., Asay, K.H., Tieszen, L.L. (1991) Carbon isotope discrimination, gas exchange, and water-efficiency in crested wheatgrass clones. *Crop Science, 31:1203-1208.*

Rusk, G.D., Pennefeather, M., Dobson, D.A.G. and Ferguson, J.B. (1991) The economics of growing Eucalyptus in the Republic of South Africa. In: Intensive Forestry: The Role of Eucalyptus. 2:870-878. IUFRO Symposium, Durban, South Africa.

Sands, P.J., Cromer, R.N. and Kirshbaum, M.U.F. (1992) A model of nutrient response in *Eucalyptus grandis* seedlings. *Australian Journal of Plant Physiology, 19:459-470.*

Schleser, G.H. and Jayaseker, R. (1985) $\delta^{13}C$-Variations of leaves in forests as an indication of reassimilated CO_2 from the soil. *Oecologia, 65:536-542.*

Schleser, G.H. (1990) Investigations of the $\delta^{13}C$ pattern in leaves of *Fagus sylvatica* L. *Journal of Experimental Botany, 41:565-572.*

Schleser, G.H. (1992) $\delta^{13}C$ Pattern in a forest tree as an indicator of carbon transfer in trees. *Ecology, 73:1933-1925.*

Schlesinger, W.H. and Hasey, M.M. (1981) Decomposition of chaparral shrub foliage: losses of organic and inorganic constituents from deciduous and evergreen leaves. *Ecology, 62(3),762-774.*

Schönau, A.P.G. (1991) Role of eucalypt plantations in timber supply and forest conservation in Sub-Saharan Africa. *South African Journal of Forestry, 156:56-60.*

Schulze, E.-D., Schilling, K. and Nagarajah, S. (1983) Carbohydrate partitioning in relation to whole plant production and water use of *Vigna unguiculata* (L.) Walp. *Oecologia, 58:169-177.*

Schulze, R.E. (1991) Global climate change and hydrological response: A Southern African perspective. In: Proceedings of the Fifth South African National Hydrological Symposium. Stellenbosch. pp1.1-1.17

Sealy, J. (1986) Stable carbon isotopes and prehistoric diets in south western Cape Province, South Africa. Cambridge Monographs in African Archaeology No 15. British Archaeological Reports, International Series No. 293, 150p.

Sheriff, D.W. and Nambiar, E.K.S. (1991) Nitrogen nutrition, growth and gas exchange in *Eucalyptus globulus* Labill. seedlings. *Australian Journal of Plant Physiology, 18:37-52.*

Sheriff, D.W. (1992) Roles of carbon gain and allocation in growth at different nitrogen nutrition in *Eucalyptus camaldulensis* and *Eucalyptus globulus* seedlings. *Australian Journal of Plant Physiology, 19:637-652.*

Smith, B.N.; Oliver, J.; and Mc Millan, C. (1976) Influence of carbon source, oxygen concentration, light intensity, and temperature on $^{13}C/^{12}C$ ratios in plant tissues. *Botanical Gazette, 137(2):99-104.*

Smith, V.R. (1980) A phenol-hyochlorite determination of ammonium-nitrogen in Kjeldahl digests of plant tissue. *Communications in Soil Science & Plant Analysis, 11:709-722.*

Sofer, Z. (1980) Preparation of carbon dioxide for stable carbon isotope analyses of petroleum fractions. *Analytical Chemistry, 52(8):1389-1391.*

Stanger, T.K. (1991) Early results in provenance / progeny trials of cold tolerant Eucalyptus in South Africa. In: Intensive Forestry: The Role of Eucalyptus. 1:318-329. IUFRO symposium, Durban, South Africa.

Toft, N.L., Anderson, J.E. and Nowak, R.S. (1989) Water use efficiency and carbon isotope composition of plants in a cold desert environment. *Oecologia, 80:11-18.*

Thornley, J.H.M. and Reynolds, J.F. (1982) A Shoot:root partitioning model. *Annals of Botany, 49:585-597.*

Troughton, J.H., Wells, P.V. and Mooney, H.A. (1974) Photosynthetic mechanisms and paleoecology from carbon isotope ratios in ancient specimens of C_4 and CAM plants. *Science, 74:298-301.*

Turnbull, J.W. (1991) Future use of Eucalyptus: opportunities and problems. In: Intensive Forestry: The role of Eucalyptus. 1:2-21. IUFRO symposium, Durban, South Africa.

van der Merwe, N.J. and Medina, E. (1989) The canopy effect, carbon isotope ratios and food webs in Amazonia. *Journal of Archaeological Science, 18:249-259.*

van Lill, W.S., Kruger, F.J. and van Wyk, D.B. (1980) The effect of afforestation with *Eucalyptus grandis* Hill ex Maiden and *Pinus patula* Schlecht. et Cham. on streamflow from experimental catchments at Mokobulaan, Transvaal. *Journal of Hydrology, 48:107-118.*

van Wyk, D.B. (1987) Some effects of afforestation on streamflow in the Western Cape Province, South Africa. *Water South Africa, 13(1):31-36.*

Vogel, J.C. (1978) Recycling of CO_2 in a forest environment. *Oecologia Plantarium, 13:89-94.*

Vogel, J.C. (1980) Fractionation of the carbon isotopes during photosynthesis. *Sitzunsberichte der Heidelberger Akademie der Wissenschaften, 3:111-135.*

von Caemmerer, S. and Evans, J.R. (1991) Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. *Australian Journal of Plant Physiology, 18:287-305.*

Vos, J. and Groenwold, J. (1989) Genetic differences in water-use efficiency, stomatal conductance and carbon isotope fractionation in potato. *Potato Research, 32:113-121.*

Waring, R.H. and Schlesinger, W.H. (1985) Forest Ecosystems: Concepts and Management. Academic Press, London. 340p

Wullschleger, S.D. and Oosterhuis, D.M. (1989) Water use efficiency as a function of leaf age and position within the cotton canopy. *Plant and Soil, 120:79-85.*

Zar, J.H. (1984) Biostatistical Analysis. Second Edition. Prentice-Hall, New Jersey. 718p

APPENDIX ONE

CALIBRATION OF NEUTRON PROBE COUNT RATIOS WITH SOIL MOISTURE CONTENT IN EXPERIMENTAL DRUMS

INTRODUCTION

The Troxler depth moisture gauge (3300 series) or "neutron probe" works on the principle of nuclear thermalization. Fast neutrons are emitted by an Americium 241:Beryllium radioactive source and are thermalized (slowed) by hydrogen in the soil sample. The slowed neutrons are detected and counts are displayed in direct proportion to the water content of the sample (Rundel and Jarrell, 1989). For the purpose of using neutron probe count ratios as a measure of soil moisture in the experimental drums, count ratios were calibrated against soil moisture content within the drums.

METHODS

Count ratio to soil moisture content calibration was performed on 10 April 1991 following the methods of Greacen (1981). A single drum that had been packed previously with soil in the field during the preparation of the field pot trial was emptied on to a plastic sheet (2.5 x 2.5 meters). Soil was allowed to dry in the sun for three days after which it was passed through a 6 mm mesh sieve. An aluminium access tube was placed in the middle of the drum on repacking of the soil up to 50 cm. Compaction of soil was performed on repacking. Three count ratio readings were taken with the probe in the centre of the soil profile. Soil was unpacked to mid-depth and three soil cores were collected with metal cylinders. Soil in cylinders were weighed immediately, the soil removed and dried in an oven at 105^0 for 48-72 hours and reweighed to obtain dry mass. Soil cylinders were weighed and subtracted from the mass of soil in cylinders to obtain wet mass of soil. Soil core lengths and widths were measured to determine soil core volume. The remaining soil was removed from the drums, spread out on the plastic sheet to approximately 5cm deep. A known volume of water was sprinkled over the soil. Soil was mixed thouroughly with a builders shovel and repacked in the drums. The process was repeated six times until a range of count ratios and soil moisture contents from sun dried soil to above saturation (expected volumetric values obtained from Christie, unpublished data) were measured. Further sampling of count ratios versus soil water content were taken from six drums in the field at 30 and 60 cm depths. These were included to increase the sample size.

Calculations for gravimetric soil moisture content, bulk density, volumetric soil moisture content and litres of water present in soil profile were as follows:

Wet soil mass, M_w = wet mass of cylinder and soil - cylinder mass (g soil and H_2O) **(1)**

Dry soil mass, M_d = mass of oven dried soil at 105^0C for 48-72 hours (grams soil) **(2)**

Gravimetric water content, $W = (M_w - M_d)/M_d$ (g $H_2O.g^{-1}$soil) **(3)**

Soil Bulk volume, $V = \pi r^2 l$ cm^3 where r is the radius and l is the length of the soil core respectively **(4)**

Bulk density, $p = M_d/V$ $g.cm^{-3}$ **(5)**

Volumetric water content, Φ = Wp g H_2O in soil bulk volume cm^3 **(6)**

Litres of water in the soil profile, L is equivalent to the mass of H_2O in the soil profile derived from the following equation:

mass of H_2O in soil = Φ ($kg.m^{-3}$) * 1000 * soil volume (m^3), **(7)**

where 1000 is the factorial increase in volumetric soil moisture when expressed as kg H_2O in soil bulk volume m^3 and soil volume in drum is determined as $\pi r^2 h$, where r is the radius and h is the height of the soil profile respectively.

RESULTS

Linear regressions between count ratios measured with the neutron probe and soil moisture content expressed as volumetric and litres were calculated using QUATRO PRO and are shown in Figures 1 a and b .

APPLICATION OF CALIBRATED COUNT RATIOS TO SOIL MOISTURE CONTENT

The significant relationships derived between count ratio and soil volumetric content and litres validated the use of the neutron probe count ratios as a measure of soil moisture content. The regression equations for count ratios versus volumetric soil water content and litres in soil were used in a QUATRO PRO programme to obtain the number of litres present in the soil profile in the drum at the respective count ratios measured on a weekly basis.

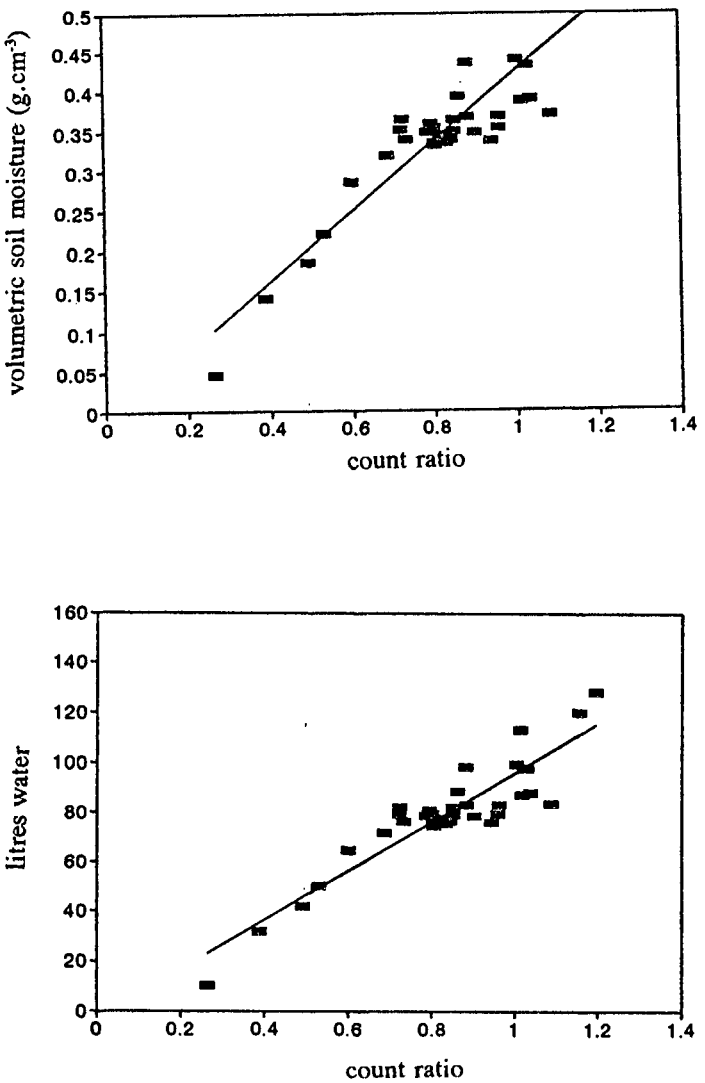


Figure 1. (a) Relationship between volumetric soil moisture versus count ratio. Volumeteric soil moisture = 0.439 neutron count ratio - 0.013. (b) Relationship between litres of water in drum and neutron count ratio. Litres of water = 99.24 countr ratio + 3.15.

REFERENCES

Greacen, E.L. (1981) Soil water assessment by the neutron method, CSIRO, East Melbourne, 140pp.

Rundel, P.W. and Jarell, W. M. (1989) Water in the environment. In: Plant Physiological Ecology. (Eds. R.W. Pearcy, J. Ehleringer, H.A. Mooney and P.W. Rundel) Chapman and Hall.

APPENDIX TWO

THE WATER RETENTION CURVE CHARACTERISTIC OF THE SOILS USED IN THE EXPERIMENT

INTRODUCTION

Soil water related studies require the estimatation of the percentage of total soil water available to the plant since even in a case where the soil water content of a soil may be high, a small proportion of the water may be available to the plant and the plant may be under stress without visible symptoms (van der Merwe, 1990). The classic upper and lower limits of available soil water for plant absorption are field capacity and permanent wilting percentage and is approximated soil water potentials of -0.03 and -1.5 MPa respectively in clay soils (van der Watt et al. 1989). Water molecules are held in the interstices between soil particles at an intensity that is related to a water potential that results from the reduction of free energy of a water molecule mainly due to surface tension and the amount of dissolved salts in it (Rundel and Jarrell, 1989). Since these physical phenomena are mainly a function of the size distribution of soil pores and the chemical nature of the soil parent material, the behaviour of water molecules would differ between soil types. For a given soil there is expected to be a unique relationship between the soil water content and the soil water potential; the relationship known as the soil water retention curve (Rundel and Jarrell, 1989). The aim was firstly, to determine the volumetric moisture content available water for plant uptake in the soil profile of the drums, and secondly, to determine the range of soil water potentials that the plants were subjected to in the two watering treatments by constructing a soil water retention curve.

METHODS

Soil cores, 3 x 5 cm, were sampled at 30 cm from ten drums at the end of the experiment. A soil retention curve was determined by quantifying the volumetric moisture content at a range of pressures from 0.05 to 1.5 MPa using the pressure plate technique (van der Merwe, 1990).

Field capacity, permanent wilting point and plant available water were determined as follows:

Feild capacity = % volumetric moisture content between 0.01 and 0.03 MPa (a)

Permanent wilting point = % volumetric moisture content at 1.5 MPa. (b)

Plant available soil water = the moisture content ranging between field capacity and permanent wilting percentage, (a) - (b).

Conversion of volumetric moisture content at field capacity and at permanent wilting point to litres were estimated using equation 7, appendix 1. This provided a means to express the range of soil moistures across which the two watering treatments spanned as a percentage field capacity.

RESULTS

Figure 1 shows the volumetric soil moisture content expressed as a percentage at a range of soil water potentials for the soil profile in the experimental pots. Mean volumetric soil moisture percentage at field capacity and permanent wilting point are estimated at 27.74% and 18.94%, respectively. The plant available soil moisture percentage in the soil profile is estimated to be approximately 8.8%.

The range of soil moisture across which the seedlings from the two watering treatments spanned are expressed as a percentage field capacity in Table 1. It was calculated that the plants in the high watering treatment were subjected to a soil moisture percentage ranging from 80 to 130% field capacity whereas the plants from the low watering treatment were subjected to soil moisture percentages ranging between 70 and 95% field capacity.

Table 1. Expression of soil moisture percentage as a percentage field capacity that the high and low watered experimental plants were subjected to on a weekly cycle.

Soil moisture parameters	High water		Low water		F.C.
	Upper limit	Lower limit	Upper limit	Lower limit	
Litres in soil profile	80	50	60	45	62.6
Volumetric moisture percentage	35.9	22.1	26.6	19.9	27.8
% F.C. = vol. measure / vol. at F.C.	130	80	95	70	100

The plants of the low watering treatment were subjected to half the range of soil moisture

that the high watered plants spanned.

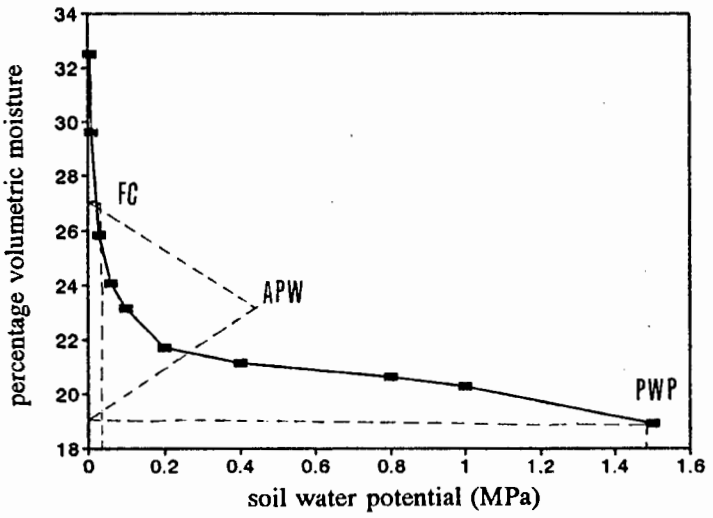


Figure 1. Soil water retention curve for the soils in the experimental drums. Field capacity (FC), available plant water (APW) and permanent wilting percentage (PWP) are depicted.

REFERENCES

Rundel, P.W. and Jarell, W.M. (1989) Water in the environment. In: Plant Physiological Ecology. (Eds. R.W. Pearcy, J. Ehleringer, H.A. Mooney and P.W. Rundel) Chapman and Hall.

van der Merwe, L. (1990) A technique for producing soil water retention curves. *South African Journal of Forestry, 155:27-29.*

van der Watt, HvH., Johnston, M.A., Mottram, R., Savage, M.J., Scholes, R.J. and Russell, D.A. (1989) Measurement of soil water status. In: Studying water in the soil-plant-atmosphere continuum: a bibliographic guide to techniques. (Eds. R.J. Scholes and M.J. Savage) South African National Scientific Programmes Report No 163. pp13-25.

Plate 1. Pot experiment with commercial clones of *Eucalyptus* in the field at the D.R. de Wet Forestry Research Centre. Leaf canopy after 10 weeks growth.

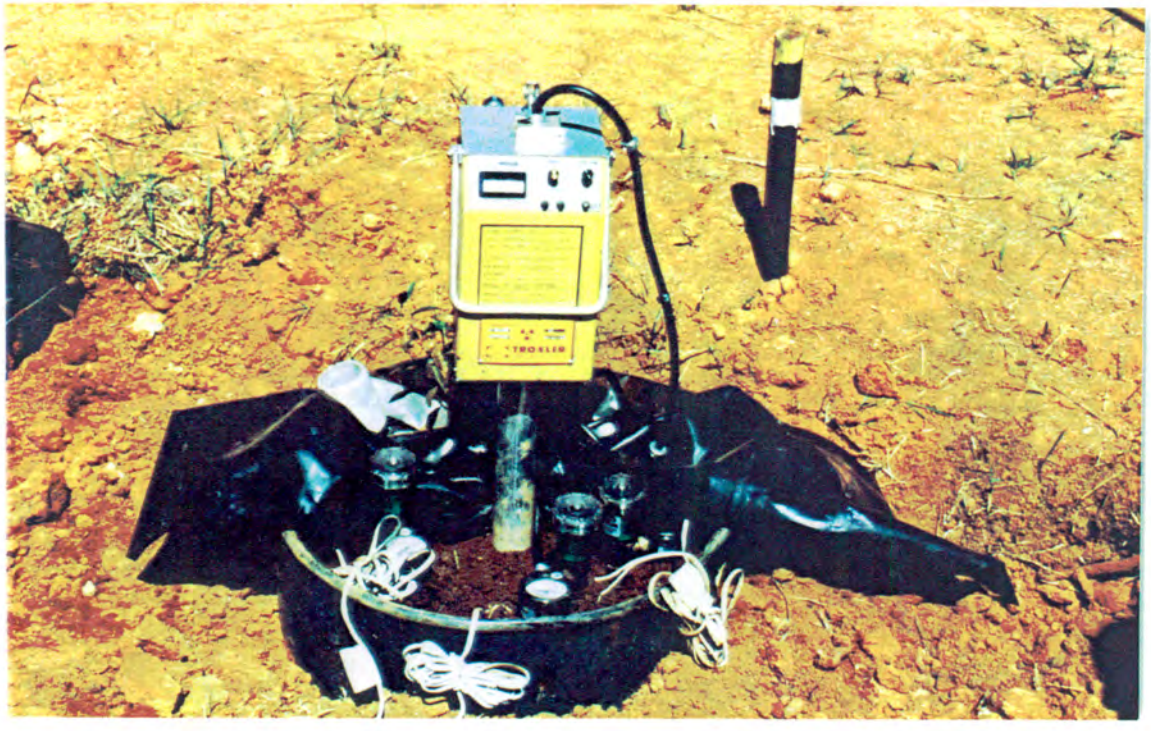


Plate 2. Measurement of soil moisture using the neutron probe.