Dry mass allocation, water use efficiency and $\delta^{13}C$ in clones of *Eucalyptus grandis, E. grandis × camaldulensis* and *E. grandis × nitens* grown under two irrigation regimes

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Summary Clonal variation in water use efficiency (WUE), dry mass accumulation and allocation, and stable carbon isotope ratio ($\delta^{13}C$) of crude leaf fiber extracts was determined in six clones of *Eucalyptus grandis* W. Hill ex Maiden. grown for 16 months in field lysimeters in two soil water regimes. The relationships between $\delta^{13}C$ and WUE calculated on the basis of leaf, harvestable stem, shoot and whole-plant dry mass accumulation were investigated. There was no clonal variation in dry mass accumulation but clonal allocation to roots, harvestable stems, branches and leaves varied. Water use efficiencies (mass of plant or plant part/water used over 16 months) differed significantly between clones. The clonal ranking of WUE varied depending on the units of dry mass accumulation used. Significant relationships between $\delta^{13}C$ values and instantaneous water use efficiencies and ratios of internal leaf to ambient CO_2 concentrations were found only in the high soil water treatment. There were no relationships between $\delta^{13}C$ values and whole-plant, shoot and harvestable stem water use efficiencies and soil water availability. Values of $\delta^{13}C$ were negatively correlated with dry mass accumulation in the low soil water treatment. At the whole-plant level, WUE was positively correlated with dry mass accumulation in the high soil water treatment. We found significant differences in $\delta^{13}C$ values between clones and the clonal rankings in $\delta^{13}C$ and WUE were maintained in both soil water treatments.

Keywords: intercellular to ambient CO_2 concentration ratio, lysimeters, soil water, stable carbon isotope ratio.

Introduction

In South Africa, afforestation with eucalypts is controversial because high water use by these species reduces streamflow. We explored the possibility of reducing water use by clonal selection using stable carbon isotope ratio ($^{13}C/^{12}C$, $\delta^{13}C$) as an indicator of water use efficiency (WUE).

In C_3 crop plants, $\delta^{13}C$ in leaf tissue is related to dry mass accumulation and whole-plant WUE (Farquhar et al. 1982, Farquhar and Richards 1984, Hubick et al. 1986, Martin and Thorstenson 1988, Vos and Groenwold 1989). This relationship is determined by, among other factors, stomatal control of the ratio of the instantaneous rates of photosynthesis (A) and transpiration (E) (Farquhar and Sharkey 1982), and the degree of discrimination against $\delta^{13}C$ with changing ratio of leaf intercellular to ambient CO_2 concentrations (c_i/c_a) (Farquhar et al. 1982, Farquhar and Richards 1984). Genetic variation in $\delta^{13}C$ and WUE (ratio of dry mass accumulation to water loss over the growing season) has been demonstrated in several crops including wheat, peanut, tomato and potato genotypes (Farquhar and Richards 1984, Hubick et al. 1986, Martin and Thorstenson 1988, Vos and Groenwold 1989, see Ehleringer et al. 1993 for review). Knowledge of the genetic basis of differences in $\delta^{13}C$ may be useful for ranking WUE among clones and species and as a guide in breeding for improved yield under drought conditions.

Recently the relationships between $\delta^{13}C$ and dry mass accumulation and WUE have been explored in woody shrub and tree species. Negative correlations between $\delta^{13}C$ of leaf tissue and tree height were demonstrated in 13-month old commercial clones of *Eucalyptus grandis* W. Hill ex Maiden. implying that less water-use-efficient trees were more productive (Bond and Stock 1990). Similarly, growing season WUE and $\delta^{13}C$ were positively correlated in western larch and *Eucalyptus globulus* Labill. seedlings (Zhang and Marshall 1993, Osório and Pereira 1994). Genetic variation in WUE and $\delta^{13}C$ was also reported for both tree and shrub species (Hubick and Gibson 1993, Zhang and Marshall 1993, Zhang et al. 1993, Donovan and Ehleringer 1994). Although clonal variation in $\delta^{13}C$ was shown in a study of four-year-old clones of *Eucalyptus grandis* (Olbrich et al. 1993), a poor relationship was found between $\delta^{13}C$ and WUE in the production of harvestable stems (i.e., the water cost of wood production) suggesting that the relationship between $\delta^{13}C$ and WUE in leaves may change when scaling up to stems, shoots and whole trees. Variation in allocation patterns in trees could result in simultaneous changes in harvestable stem coupled with changes in WUE. Individuals allocating a high proportion of dry mass to stems could have a

high WUE when expressed on harvestable stem basis but have a low WUE at the whole-plant level.

We investigated the relationship between $\delta^{13}C$ and WUE at various levels of tree organization to determine the value of $\delta^{13}C$ for screening for WUE and improvement of yield under drought conditions in *Eucalyptus* clones. Specifically, we tested two hypotheses: (1) clonal variation in dry mass accumulation and allocation, water use efficiency and $\delta^{13}C$ of *Eucalyptus* clones grown under two water regimes can be detected at the juvenile stage of development; (2) there are significant relationships between leaf $\delta^{13}C$ values, instantaneous WUE (WUE_i), harvestable stem WUE (WUE_h), shoot WUE (WUE_{sh}), whole-plant WUE (WUE_p) and whole-plant dry mass accumulation in *Eucalyptus* clones grown at two water regimes.

Methods

Ten replicate cuttings of six *Eucalyptus* clones (Clone 1 = *E. grandis* × *camaldulensis* (GXC 011/025), Clone 2 = *E. grandis* (JDM 254/005), Clone 3 = *E. grandis* (KFT 023/033), Clone 4 = *E. grandis* (JDM 063/002), Clone 5 = *E. grandis* × *nitens* (GXN 191/401), and Clone 6 = *E. grandis* × *nitens* (GXN 360/148)) were grown in 225-l drums (60 cm wide × 80 cm high) in the field at the D.R. de Wet Forestry Research Station, near Sabie, South Africa (25°3′ S, 30°53′ E) from April 1991 until July 1992. Rainfall was excluded from the drums by means of plastic lids that allowed the plant stem and leaf canopy to protrude. Soil water was measured with a neutron probe head lowered into centrally placed access tubes (Troxler Depth Moisture Gauge, Model 3300, Troxler Electronic Laboratories, Research Triangle Park, NC), previously calibrated against volumetric soil water. Half of the cuttings were subjected to a high soil water treatment and the remaining cuttings were subjected to a low soil water treatment so that soil water in the drums was maintained at 100 and 80% of field capacity, respectively, on a weekly basis. At 15 months, WUE_i and c_i/c_a were measured once on five leaves of three trees of each clone and each treatment with an LCA2 infrared gas analyzer, air supply unit and narrow Parkinson leaf chamber (Analytic Development Co. Ltd., Hoddeson, England). Net photosynthetic rate, stomatal conductance and transpiration rate were measured at > 1200 μmol m^{-2} s^{-1}, which is above the saturation point for *Eucalyptus grandis* leaves (unpublished results). Leaf chamber conditions tracked ambient air temperatures ranging between 19 and 34 °C and the chamber CO_2 concentration was approximately 350 ppm over the 5-day measuring period. Because water was removed from air entering the leaf chamber by means of calcium sulfate traps, the leaf was exposed to dry air for 1 min while measurements were made. Ambient atmospheric relative humidities at the study site measured with an Asman psychrometer at hourly intervals during the period that photosynthetic measurements were made were low (27%, SD = 10.2, $n = 28$). Thirty, healthy, fully expanded leaves were sampled from the north side at approximately two thirds of the length of the canopy (1.4 m above ground) from three trees of each clone and each treatment for

$\delta^{13}C$ analyses. Two weeks later, the plants were felled at 5 cm above the soil surface and separated into leaves, branches and main stems. Roots were separated from soil by dry sieving through a 10-mm sieve. 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 h. Total plant (WUE_p), shoot (WUE_{sh}) and harvestable stem (WUE_h) water use efficiencies were expressed as grams dry mass accumulated for the respective plant parts per liter of water used over the growing season.

The $^{13}C/^{12}C$ ratios were measured on carbon dioxide samples derived from crude leaf fiber extracts (Schlesinger and Hasey 1981, Brugnoli et al. 1988, Le Roux 1993), with a Micromass Micromass 602E dual inlet, double collector mass spectrometer (VG, Middlewich, Cheshire, UK), where the reference gas was calibrated against six National Bureau of Standards isotopic reference materials, NBS 16, 17, 18 19, 20 and 21. The ratios were expressed as $\delta^{13}C$ against a Chicago Pee Dee Belemnite marine limestone standard, where $\delta^{13}C = [(R \text{ sample}/R \text{ standard}) - 1] \, 1000$, and $R = {}^{13}C/{}^{12}C$. Reproducibility was 0.1‰ with well-homogenized glucose standards.

Statistical analyses, data manipulation and graphic display were performed with MINITAB (Version 8.2, Minitab Inc, PA, USA), QUATRO PRO (Version 4.0, Borland International Inc., CA, USA) and AXUM (Version 1.0, Trimetrix Inc, WA, USA) software packages. Two-way and one-way ANOVAs were performed to determine the effects of water availability and genotype on the variables after which multiple comparisons among means were made by Fisher's Least Significant Difference Multiple Range Test (Sokal and Rolf 1984).

Results

Clonal variation in dry mass accumulation, dry mass allocation, water use efficiency and $\delta^{13}C$

After 16-months, there were no significant differences ($P < 0.05$) among clones in dry mass accumulation in either soil water treatment ($F_{5,12} = 0.418$ and 2.921, respectively) (Table 1). However, there were significant differences among clones in dry mass allocated to roots, harvestable wood, branches and leaves (Figures 1a and 1b) in both soil water treatments. Clones 3 and 4 invested a larger proportion of dry mass in roots than the other clones ($F_{5,12} = 7.8$ and 6.8 respectively, $P < 0.05$), whereas Clones 5 and 6 invested a larger proportion of dry mass in leaves than the other clones ($F_{5,12} = 13.0$ and 17.7 respectively, $P < 0.05$). Clone 1 preferentially allocated dry mass to harvestable stem ($F_{5,12} = 12.9$ and 6.7 respectively, $P < 0.05$) at the expense of allocations to branches ($F_{5,12} = 9.9$ and 5.0 respectively, $P < 0.05$) and leaves in both soil water treatments. There were significant treatment effects on clonal responses in allocation of dry mass to harvestable stem ($F_{5,12} = 2.96$, $P < 0.05$). In response to the low soil water treatment, Clones 2 and 4 increased allocation to harvestable stem, whereas the other clones decreased allocation to harvestable stem.

Water use efficiencies differed significantly between clones (Figure 2, Table 1), and clonal rankings were not affected by

Table 1. Mean water use efficiency calculated on the basis of whole plant (WUE_p), shoot (WUE_{sh}) and stem wood (WUE_h) dry matter accumulation over 16 months and stable carbon isotope ratio ($\delta^{13}C$) of crude leaf fiber extracts in clones of *Eucalyptus* grown at two soil water availabilities. Values are means and standard errors for the soil water treatments combined. Different letters indicate means are significantly different at $P < 0.05$ (Fisher's Least Significant Difference Multiple Range Test after two-way analysis of variance).

Clone	Dry mass (g)	Mean WUE_p (g l^{-1})	Mean WUE_{sh} (g l^{-1})	Mean WUE_h (g l^{-1})	Mean $\delta^{13}C$ (‰)
1	1601 a ± 155	2.90 a ± 0.16	1.35 ab ± 0.11	0.75 d ± 0.05	−25.75 a ± 0.73
2	1532 a ± 308	3.17 a ± 0.11	1.50 b ± 0.13	0.64 c ± 0.04	−25.30 a ± 1.82
3	1669 a ± 237	3.04 a ± 0.12	1.15 a ± 0.06	0.53 b ± 0.02	−25.44 a ± 1.26
4	1612 a ± 297	3.10 a ± 0.20	1.21 a ± 0.13	0.42 a ± 0.05	−25.81 a ± 1.01
5	1329 a ± 238	2.95 a ± 0.21	1.58 bc ± 0.18	0.51 ab ± 0.05	−24.64 b ± 0.48
6	1490 a ± 241	3.62 b ± 0.25	1.99 d ± 0.18	0.70 d ± 0.03	−24.77 b ± 0.87

the soil water treatments (Table 2). Among clones, Clone 6 exhibited the highest water use efficiency in both soil water treatments. Because of clonal variation in dry mass allocation patterns, the clonal ranking in water use efficiency was sensitive to the measures of productivity used. Clone 1 had the lowest WUE_p and the highest WUE_h, because it allocated more dry mass to harvestable stem. As a result of the significant differences in dry mass allocation between clones, clones differed significantly with respect to the water cost of wood production. Clones 1 and 6 used approximately 1000 dm^3 less water than Clone 4 to produce 1 kg of harvestable stem (derived from Table 1).

Clones differed significantly in $\delta^{13}C$ of leaf fiber extracts and the ranking was maintained across treatments (Tables 1 and 2). Clone 6 had $\delta^{13}C$ values about 1‰ more enriched than Clone 1. This shift in $\delta^{13}C$ corresponded with a 20% increase in WUE_p. Clonal variation in $\delta^{13}C$ values paralleled clonal variation in WUE_{sh} (Table 1). Clones 5 and 6 had significantly higher WUE_{sh} values and significantly less negative $\delta^{13}C$ values than Clones 1 and 4. Despite the low WUE_p and more negative $\delta^{13}C$ values exhibited by Clone 1, this clone had the highest WUE_h (Table 1).

Relationships between $\delta^{13}C$ and c_i/c_a, WUE_i, WUE_h, WUE_{sh}, WUE_p and productivity

Significant correlations ($P < 0.05$) were found between WUE_i and $\delta^{13}C$ and c_i/c_a among individuals in the high soil water treatment, whereas no significant correlations were found be-
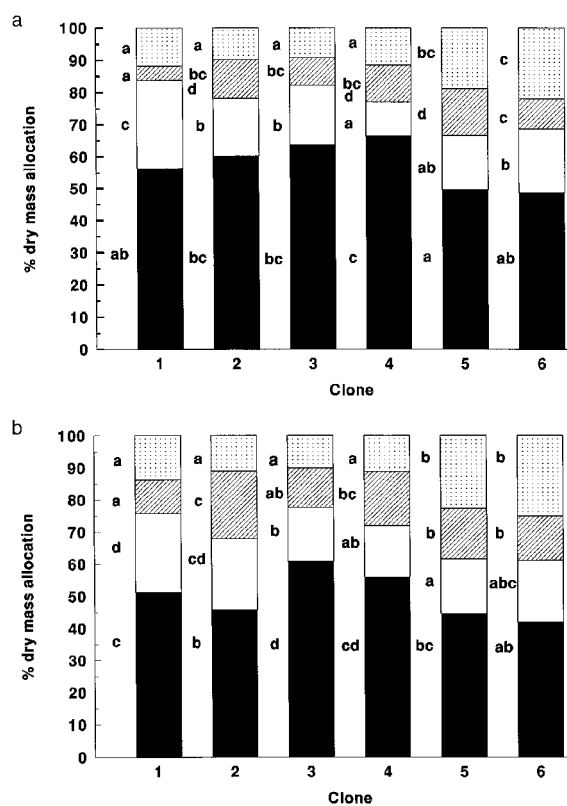
Figure 1. Relative mean clonal allocation to roots (filled), main stems (empty), branches (hatched) and leaves (dotted) for high (a) and low (b) soil water treatments of 16-month-old clones of *Eucalyptus*. Different letters indicate significant differences among clones at each organ level (Fisher's Least Signficant Difference Multiple Range Test following one-way ANOVA of arcsine transformed data, $P < 0.05$).
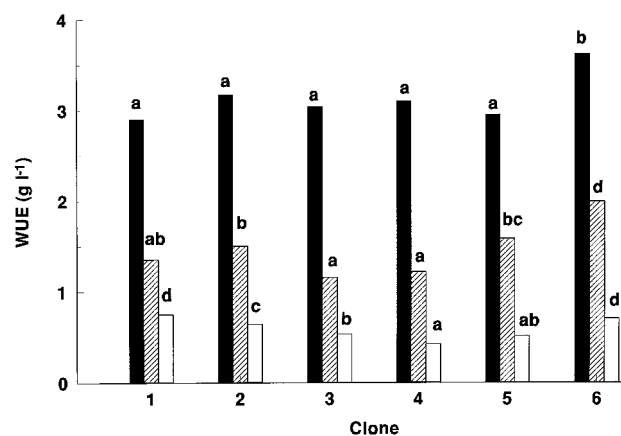
Figure 2. Mean clonal total plant (WUE_p) (filled), shoot (WUE_{sh}) (hatched) and harvestable stem (WUE_h) (empty) water use efficiencies for 16-month-old *Eucalyptus* clones. Different letters indicate significant differences among clones (high and low water treatments combined) (Fisher's Least Significant Difference Multiple Range Test with significance at $P < 0.05$).

Table 2. The F values derived from Model I two-way analysis of variance to test the significance of clone ($n = 6$) and water treatment ($n = 2$) on whole plant (WUE_p), shoot (WUE_{sh}) and harvestable stem (WUE_h) water use efficiency. An asterisk denotes a significant difference at $P < 0.05$.

Variable	Clonal effect	Treatment	Interaction
WUE_p	2.8^*	14.5^*	0.6^{NS}
WUE_{sh}	10.3^*	36.0^*	0.5^{NS}
WUE_h	14.5^*	17.7^*	1.2^{NS}
$\delta^{13}C$	3.3^*	52.5^*	1.7^{NS}
Dry mass	0.9^{NS}	85.8^*	0.8^{NS}

tween WUE_i and $\delta^{13}C$ among individuals from the low soil water treatment (Figure 3a and 3b). High WUE_i and less negative $\delta^{13}C$ values of leaf fiber extracts were associated with low c_i/c_a ratios. There were no significant correlations between

$\delta^{13}C$ values of leaf fiber extracts and WUE_p in either treatment (Figure 4). Similarly, $\delta^{13}C$ of leaf fiber extracts was not significantly correlated with WUE_{sh} ($r = 0.28$ and 0.41, $n = 18$) and WUE_h ($r = 0.32$ and $r = 0.23$, $n = 18$) in either treatment (data not shown). Whole-plant dry mass accumulation was significantly negatively correlated with $\delta^{13}C$ in the low soil water treatment, but this negative correlation was not significant in the high soil water treatment (Figure 5a). There was no significant correlation between dry mass accumulation and WUE_p in the low soil water treatment, whereas dry mass accumulation correlated positively with WUE_p in the high soil water treatment (Figure 5b).

Discussion

We found large differences in clonal dry mass allocation to above- and belowground parts and harvestable stem dry mass among eucalypt clones after 16 months of growth. Allocation to harvestable stem was affected by availability of water in some clones, implying that economic yield may be improved under conditions of limited water availability in certain genotypes. Although whole-plant dry mass accumulation did not differ significantly between genotypes, genotypes differed significantly with respect to growth season water use efficiencies and $\delta^{13}C$ as has been found for other trees and shrubs (Zhang et al. 1993, Zhang and Marshall 1993, Osório and Pereira 1994, Donovan and Ehleringer 1994a and 1994b).

In the high soil water treatment, the relationship between $\delta^{13}C$ and WUE at the leaf level supported the model predictions of Farquhar and Richards (1984), whereas this was not the case for plants in the low soil water treatment. The lack of a significant relationship between WUE_i and c_i/c_a and $\delta^{13}C$ in the low soil water treatment was mainly due to stomatal closure ($c_i/c_a > 1$) in some clones in response to the low ambient relative humidities experienced during the measurement pe-
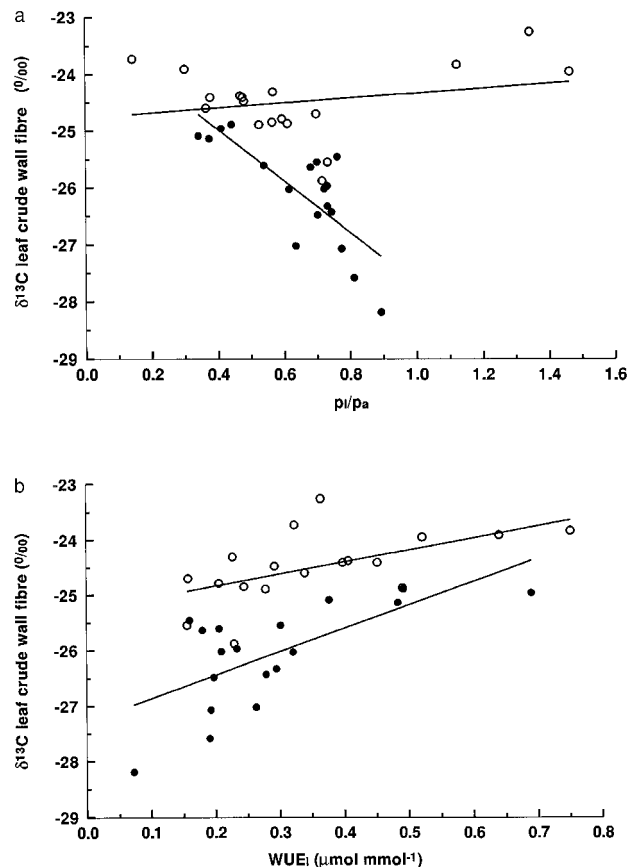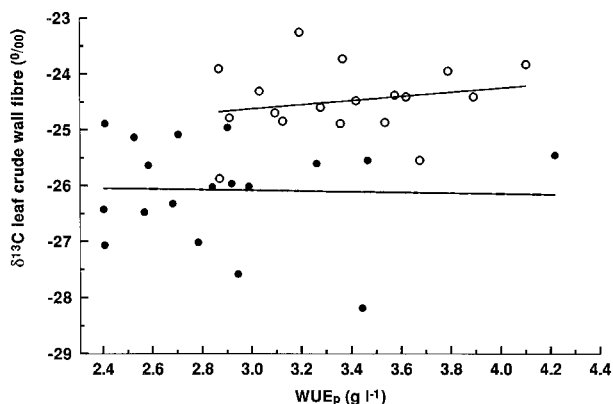

Figure 3 (a) Relationship between $\delta^{13}C$ of crude fiber extracted from leaves of 16-month-old *Eucalyptus* clones and c_i/c_a for the high (●) and low (○) soil water treatments. $\delta^{13}C = 4.46\ c_i/c_a$, $r = 0.77$, $n = 18$, $P < 0.05$ for the high soil water treatment. (b) Relationships between $\delta^{13}C$ of crude fiber extracted from leaves of 16-month-old *Eucalyptus* clones and instantaneous water use efficiency for the high (●) and low (○) soil water treatments. $\delta^{13}C = 4.23\ WUE_i\ 27.3$, $r = 0.63$, $n = 18$, $P < 0.05$ for the high water treatment. $\delta^{13}C = 1.29\ WUE_i\ 25.0$, $r = 0.40$, $n = 18$, $P > 0.05$ for the low water treatment.

Figure 4. Relationships between $\delta^{13}C$ of crude fiber extracted from leaves of 16-month-old *Eucalyptus* clones and total plant WUE (WUE_p) for high (●) and low (○) soil water treatments. $\delta^{13}C$ CWF = $0.09\ WUE_p\ -25.8$, $r = 0.04$, $n = 18$, $P > 0.05$ for the high water treatment. $\delta^{13}C$ CWF = $0.375\ WUE_p\ -25.8$, $r = 0.21$, $n = 18$, $P > 0.05$ for the low water treatment.
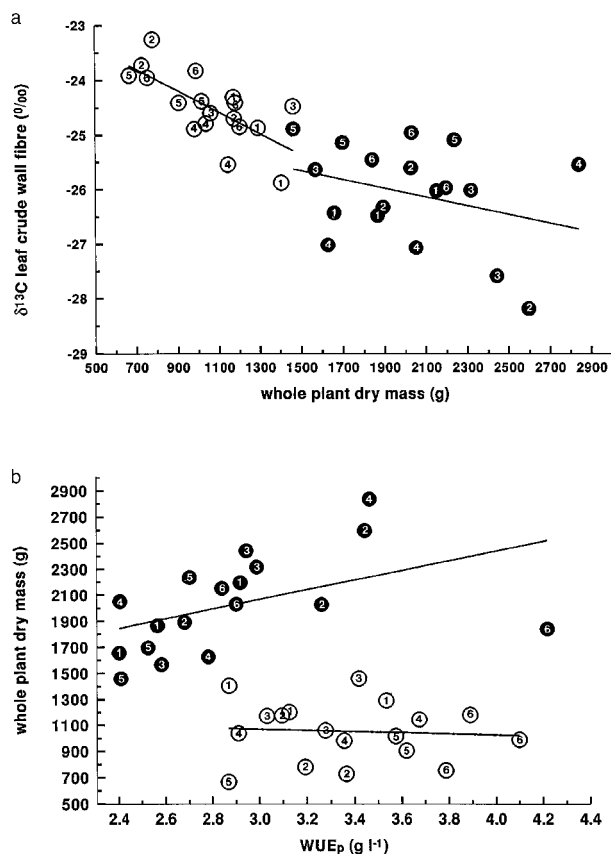
Figure 5 (a) Relationships between $\delta^{13}C$ of crude fiber extracted from leaves of 16-month-old *Eucalyptus* clones and plant dry mass for the high (●) and low (○) soil water treatments. $\delta^{13}C = -0.0008$ dry mass -24.5, $r = 0.32$, $n = 18$, $P > 0.05$ for the high soil water treatment. $\delta^{13}C = -0.002$ dry mass -22.4, $r = 0.70$, $n = 18$, $P < 0.05$ for the low soil water treatment. (b) Relationships between plant dry mass and total plant WUE (WUE$_p$) over 16 months for the high (●) and low (○) soil water treatments. Dry mass $= 373$ WUE$_p$ $+ 949$, $r = 0.47$, $n = 18$, $P < 0.05$ in the high soil water treatment. Dry mass $= 1199 - 44$ WUE$_p$, $r = 0.07$, $n = 18$, $P > 0.05$ in the low soil water treatment.

riod. However, there were no relationships between WUE$_p$, WUE$_{sh}$, WUE$_h$ and $\delta^{13}C$ in either treatment. This contrasts with the findings of Martin and Thorstenson (1988) who showed that variation in $\delta^{13}C$ in tomato leaves could explain 57 to 65% of the variability in WUE$_p$ under a range of soil water availabilities similar to that used in our study. Significant correlations between carbon isotope discrimination, instantaneous and growing season water use efficiencies were also found in western larch seedlings (Zhang and Marshall 1993). As reported for Douglas-fir and *Eucalyptus grandis* and *E. grandis* × *camaldulensis* clones (Zhang et al. 1993, Bond and Stock 1990), we found that $\delta^{13}C$ was negatively correlated with dry mass accumulation in the low soil water treatment, implying that photosynthesis was controlled by stomatal limitation for most of the growing season; however, this relationship was not significant in plants growing in the high soil water treatment. Instead, contrary to theoretical predictions, dry mass accumulation was positively correlated with WUE$_p$ in the high soil

water treatment but the correlation was not significant in the low soil water treatment. Donovan and Ehleringer (1994) reported a similar positive correlation between plant size and WUE for the desert shrub, *Chrysothamnus nauseosus* (Pall.) Britt. and suggested that WUE was not controlled by stomatal limitation in this woody perennial. Farquhar et al. (1988) suggested that a positive correlation between WUE and dry mass accumulation may occur in response to increasing stomatal conductance if the photosynthetic capacity is large and the boundary layer conductance is small. This condition may occur in dense leaf canopies where conditions within the canopy become moister and cooler thereby reducing the rate of transpiration with increasing stomatal conductance. In our study, the six genotypes investigated exhibited different canopy architectures. Clone 1 had an open canopy and few but widely spread leaves compared with Clone 6, which exhibited the largest WUE at the whole-plant level and was characterized by a dense leaf canopy (Figures 1 and 2). It is possible that, in Clone 6, the large WUE at the whole-plant level was achieved through higher canopy photosynthetic rates and lower transpiration rates. Comparisons made between WUE$_p$, WUE$_i$ and $\delta^{13}C$ among trees displaying different canopy architectures may introduce a large variance component in WUE$_p$ that is not explained by relationships with WUE$_i$ and $\delta^{13}C$.

We found different relationships between WUE$_p$ and dry mass allocation in commercial clones of *Eucalyptus* grown in two soil water treatments. In the high soil water treatment, the relationship between WUE$_p$ and dry mass accumulation was only partially dependent on leaf-level stomatal limitation of rates of photosynthesis and transpiration, suggesting that it may depend on other factors such as canopy architecture and respiratory costs associated with non-photosynthetic tissue. In contrast, stomatal limitation had a significant effect on dry mass accumulation in the low soil water treatment. However factors controlling WUE$_p$ remain to be determined. The large variability in WUE at the whole-plant and harvestable stem levels was not explained by the variability in $\delta^{13}C$, indicating that $\delta^{13}C$ may not be a reliable indicator of WUE or water cost of harvestable stem. However, the finding that clonal variation in $\delta^{13}C$ is associated with variation in WUE, and that clonal ranking in $\delta^{13}C$ is the same at both soil water availabilities tested, suggests that $\delta^{13}C$ of leaf fiber extracts may be used together with other techniques to screen for WUE.

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