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Water deficits are more important in delaying growth than in changing patterns of carbon allocation in *Eucalyptus globulus*

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Summary Potted cuttings of three *Eucalyptus globulus* Labill. clones (AR3, CN44, MP11) were either well watered or subjected to one of two soil water deficit regimes for six months in a greenhouse. Reductions in lateral branching, leaf production and leaf expansion were the leading contributors to the large differences observed in biomass production between well-watered and water-stressed plants. Although no significant differences among clones were observed in dry matter accumulation or in the magnitude of the response to soil water deficits, sensitivity of lateral branching, leaf initiation and whole-plant foliage to water stress was significantly lower in CN44 than in AR3 and MP11. When the confounding effect of differences in plant size resulting from the different watering regimes was removed, allometric analysis indicated that the genotypes differed in biomass allocation patterns. In addition to a drought-induced reduction in leaf number, water deficits also resulted in smaller leaves because leaf expansion was inhibited during dehydration events. Resumption of leaf expansion following stress relief occurred in all of the clones, but was particularly evident in severely stressed plants of Clone AR3, possibly as a result of the osmotic adjustment observed in this genotype.

Keywords: acclimation, allocation, allometry, leaf elongation rate (LER), osmotic adjustment, plant growth, water relations, water stress.

Introduction

Effects of water deficits on plant growth and metabolism have been extensively reviewed (e.g., Jones 1980, Hsiao and Bradford 1983, Schulze 1986, Chaves 1991). At the whole-plant level, limited soil water supply may have a strong effect on development, activity, and duration of various source and sink organs (Jordan 1983). Soil water deficits have been observed to cause reductions in total dry matter, lateral branching, leaf production, and rates of leaf and shoot expansion in both herbaceous and woody plants. Furthermore, many species exhibit changes in partitioning in favor of the structures involved in water uptake and transport, and increases in water use efficiency in response to water deficits (Pereira and Chaves 1993).

One question remains, however: do water deficits merely slow down growth or do they change patterns of allocation and, therefore, allometric relationships? Treatments that alter growth and development can produce effects that are prone to misinterpretation when plants of different sizes, but of the same chronological age, are compared (Coleman et al. 1993, Gebauer et al. 1996). It is important, therefore, to analyze growth in such a way that the direct effects of water deficits on allocation patterns are distinguished from the indirect effects of water availability on rates of growth and development (cf. Ledig et al. 1970, Gebauer et al. 1996).

A great diversity of techniques and experimental designs have been used to study the effects of limited water availability on plant growth (McDonald and Davies 1996), and widely different responses, or even conflicting results, have been obtained depending on how the water stress was imposed (short-term/rapid versus long-term/gradual). Most woody plants can withstand periods of soil water deficits. Under field conditions, because of the deep rooting of woody plants or the rainfall regime or both, soil water depletion generally occurs slowly during the spring and early summer months. As a consequence, unless soil water deficits are severe and persist for a long period, woody perennials usually suffer only moderate water deficits and acclimate to water shortage (Pereira and Chaves 1993). This acclimation involves changes in plant structure and function that lead to an enhancement of the plant's ability to avoid dehydration, e.g., an increase in the ratio of root biomass to leaf biomass, osmotic adjustment and stomatal closure. Thus it is important to study the consequences of both moderate and severe water deficits in trees that are allowed to dehydrate at a rate comparable with the rate of water depletion under field conditions.

We have compared the effects of two soil water deficit regimes on growth of three *Eucalyptus globulus* Labill. clones. Specifically, we studied the effects of prolonged water deficits on acclimation and on the processes of biomass allocation among functionally different plant parts. We used clonal plant material because it provides an effective way of reducing variability among replicates within treatment groups relative to the variability among treatments, thereby increasing the power and sensitivity of the experiments (Burr and Tinus 1996).

Materials and methods

Plant material and growth conditions

Rooted cuttings of three *Eucalyptus globulus* clones (CN44, AR3 and MP11) were obtained from SOPORCEL, Lisbon, Portugal. At the third leaf-pair stage, the rooted cuttings were transplanted (one plant per pot) to 10-l plastic pots filled with a 3:1:1 (v/v) mixture of soil, fine sand and peat and placed in a naturally lit greenhouse at the Instituto Superior de Agronomia, Universidade Técnica de Lisboa, in Lisbon, Portugal (39°02′ N, 09°15′ W). The pots were randomly arranged on a bench and periodically rotated to minimize effects of environmental heterogeneity. One week after transplanting, 10 g of slow-release fertilizer (Osmocote Plus, 15% N, 11% P, 13% K and 2% Mg; Grace Sierra Co., Milpitas, CA) was added to each pot. For the duration of the experiment (December 6, 1994 to June 4, 1995), the cuttings were grown in a 14-h photoperiod supplemented with artificial light from 400-W high pressure sodium lamps, to give a maximum photon flux density of photosynthetically active radiation (PPFD) of 800 μmol m^{-2} s^{-1}. Over the experimental period, day temperatures ranged from 15 to 31 °C, night temperatures ranged from 11 to 16 °C, and the relative humidity ranged from 30 to 85%.

Watering regimes

The experiment comprised nine treatments of three clones × three watering regimes in a factorial design. Ten pots of each clone were watered to field capacity and thereafter they were supplied weekly with an amount of water equal to transpirational losses (control, HW). Another ten pots were watered once per week with only 25% of the water needed to maintain the soil at field capacity (moderate plant water stress, MS). A final set of ten pots were watered once every two weeks with only 25% of the water needed to maintain the soil at field capacity (severe plant water stress, SS). Immediately before the beginning of the experiment, each pot was saturated with water and allowed to drain completely before sealing the drain hole with a rubber stopper. Evaporation from the soil surface was prevented by enclosing the pots in white plastic bags tied to the stems of the plants. The plastic cover was provided with aeration holes and, although the O_2 concentration in the soil air spaces was not measured, inspection of soil and root systems at the time of harvesting showed no signs of soil anaerobiosis. Pots were weighed to the nearest gram and initial weight was assumed to be equivalent to the weight at field capacity. Differences in pot weight between successive watering operations were used to calculate water use of the plants over the experimental period. To maintain the soil water potential of the water-stressed plants as uniform as possible, half of the required amount of water was added through an access plastic tube inserted into the soil and the other half was applied to the soil surface.

Leaf water relations

Mature leaves that had just reached full expansion were sampled from control and water-stressed plants for tissue water relations measurements. Leaf water potential (Ψ_w) was measured at predawn and at midday in four plants per treatment (one leaf per plant), with a pressure chamber (PMS Instruments Co., Corvallis, OR). Relative water content (RWC) was determined by the method of Barrs and Weatherley (1962), using a set of six discs (8-mm diameter) from each leaf (one leaf per plant and four plants per treatment). Osmotic potential (Ψ_π) measurements were made on 6-mm diameter discs by thermocouple psychrometry, using C-52 sample chambers connected to a Wescor HR-33T microvoltmeter (Wescor Inc., Logan, UT). The thermocouple psychrometers were calibrated at least once per week with standard NaCl solutions. The six discs obtained from each leaf were quickly frozen in liquid nitrogen and stored at –80 °C until analyzed. After thawing, and following an equilibration period of about 2 h, osmotic potential was measured by the dew-point method. The prevailing room temperature during the measurements was 20 ± 1 °C. Leaf turgor potential (Ψ_P) was calculated by subtracting Ψ_π from Ψ_w. To eliminate the effect of passive tissue dehydration, osmotic potentials were extrapolated to 100% of the relative water content. Osmotic potential at full turgor (Ψ_π^{100}) was calculated as: $\Psi_\pi^{100} = (\Psi_\pi \mathrm{RWC})/100$.

Leaf growth

Nondestructive measurements of leaf expansion were obtained by making positive images of the leaf blades on strips of diazo paper (Kershaw and Larsen 1992). Positive images were recorded every 3 days on three plants per treatment (one stem leaf per plant), from Day 119 to Day 160. Recordings began when the leaf had reached a sufficient size for successful application of the technique and continued until full leaf expansion. Diazo images were analyzed with a digital, camera-based, image-analysis system (Olympus CUE-2, Olympus Optical Co. Ltd., Tokyo, Japan). A reparameterized form of the conventional Richards function (Cromer et al. 1993) was fitted to the data obtained from individual leaves using the Marquardt-Levenberg algorithm:

$$S(t) = S_x[1 + rd\exp(1 + d)(t_o - t)]^{-1/d},$$

where $S(t)$ = leaf area (cm^2) at the time t (days); S_x = asymptotic (final) value of leaf area; t_o = time at which $S(t)$ undergoes its point of inflexion; r = relative growth rate of $S(t)$ at t_o ($days^{-1}$) and d is a parameter that determines the shape of the curve so that the point of inflexion occurs further up the curve with larger d. This model provided geometrically meaningful parameters that could be linked to the dynamics of leaf growth. Mean leaf expansion rates (LER, mm h^{-1}) were computed over the 3-day period between two nondestructive measurements of leaf growth.

One-milligram samples of leaf dry matter were analyzed for %N with a Europa Scientific ANCA-SL Stable Isotope Analysis System (Europa Scientific Ltd., Crewe, U.K.).

Growth analysis

Plants were harvested 180 days after the beginning of the treatments. Final shoot height and the numbers of lateral branches, leaf pairs in the stem and leaf pairs on the branches

of each seedling were recorded. Shoots were separated into stem, lateral branches, stem leaves and branch leaves. Roots were gently washed and carefully separated from soil and other debris. Plant components were then dried for at least 48 h at 70 °C in a forced draught oven and cooled in a desiccator for dry mass determination. Areas of leaves on stems and branches of each seedling were obtained with an electronic planimeter (LI-3000A, Li-Cor Inc., Lincoln, NE).

Allometric relationships between plant components or between a particular component and total biomass of the ramets were analyzed by the general model:

$$\log_e y = b_0 + b_1 \log_e x,$$

where (x, y) refer to the selected pair of dimensions and the slope coefficient b_1 represents the relative change in allocation between components with treatments.

Data analysis

Data were subjected to one- or two-way analysis of variance (ANOVA). Variables were tested for normality and homogeneity of variances and transformations made as necessary to meet the underlying statistical assumptions of ANOVA. All pairwise comparisons of individual means were done by the Bonferroni t-test and multiple comparisons against a single control group by the Dunnett's test. Differences were considered significant at $P \leq 0.05$.

To investigate whether genotype and soil water availability directly influenced patterns of dry matter partitioning between plant components or whether the differences observed reflected only size-dependent shifts in allocation, a stepwise analysis of covariance (ANCOVA) was undertaken (Gebauer et al. 1996) to remove the overall effect of plant size before testing the significance of the interactions between clone or watering regime and $\log_e x$. The presence of a significant interaction between a variable and $\log_e x$ indicates that the variable directly affected biomass allocation. When the ANCOVA showed that the slopes differed with different values of the variable, the Tukey-Kramer method was used to determine differences between the b_1 values (Sokal and Rohlf 1981).

Results

Leaf water relations

On Day 164 (when all plants were at the midpoint of a watering interval), predawn Ψ_w was significantly lower in water-stressed plants of the three clones (–0.97 ± 0.03 MPa) than in the well-watered controls (–0.34 ± 0.01 MPa). However, only in Clone MP11 was predawn Ψ_w significantly different between SS- and MS-treated plants (–1.18 versus –0.94 MPa, respectively).

Growth and dry matter partitioning

The treatments had a marked effect on the final size of ramets of all genotypes (Figure 1 and Table 1). Total biomass was reduced by soil water deficits in all three clones, with MS- and SS-treated plants exhibiting, on average, 52 and 65%, respectively, of the total dry weight accumulation of the control plants (Figure 1). Most of the drought-induced decrease in total dry weight could be accounted for by decreases in biomass allocated to branches and branch leaves. For instance, in Clones AR3 and MP11 in the MS regime, dry matter allocated to branches and branch leaves was only about 35% of that allocated to branches and branch leaves of the HW control plants, and the corresponding value for plants in the SS regime was 25% (Figure 1). Within a treatment, mean total biomass did not differ significantly among clones (Figure 1).

The water-stress treatments significantly decreased plant height (Table 1), and the decrease was less for Clone AR3 than for the other clones in both water-stress treatments (Clone AR3: –13% in MS and –26% in SS versus –19% and –33%, respectively, for Clones CN44 and MP11). However, the primary effects of soil water deficits on growth were inhibition of lateral branching and reduction of branch foliage (cf. Figure 1 and Table 1). The importance of these effects increased with the severity of the stress treatment, and Clones AR3 and MP11 were more severely affected than Clone CN44 (Table 1). Total plant leaf area (Figure 2) was reduced by 50 and 65% by the MS and SS treatments, respectively, as a result of reductions in mean area per leaf, production of new leaves and branching. The reduction in leaf size was 1.5 to 2.9 times greater for leaves on branches than for leaves on the stem axis. There was a highly significant positive correlation between total dry matter (DM_{tot}, g) and total leaf area (LA_{tot}, cm^2) ($DM_{tot} = -1.116 + 0.011\ LA_{tot}$; $R^2 = 0.94$, $P < 0.001$). Mean nitrogen concentration of leaf tissues of water-stressed plants (27 mg g^{-1}) was significantly higher (35%) than the leaf nitrogen concentration of well-watered plants (20 mg g^{-1}). Therefore, the drought-induced-limitation in dry matter production and leaf growth cannot be ascribed to reduced N acquisition associated with water shortage.

The ratios for component biomass to total biomass are shown in Table 2. By the end of the experiment, the water-stress treatments had significantly decreased the proportion of

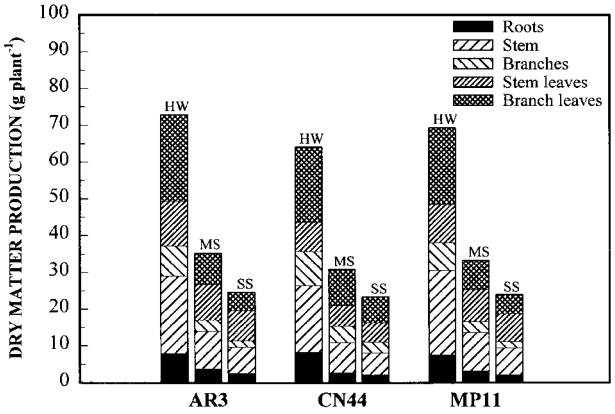

Figure 1. Effects of three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) on dry matter allocation to roots, main stems, branches and leaves by three *E. globulus* clones. Each value is the mean for six plants.

Table 1. Effects of three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) on shoot height, number of branches, number of leaf pairs and mean area per leaf of three *E. globulus* clones. Data are for the final harvest (Day 180). Each value is the mean for six plants ± standard error of the mean. For each clone and variable, a single factor analysis of variance was performed between water treatments. Means followed by the same letters are not significantly different at $P < 0.05$ (Bonferroni test). Asterisks *** significance at $P = 0.001$; ns = nonsignificant at $P = 0.05$.

	Shoot height (cm plant^{-1})	Branches (plant^{-1})	Leaf pairs (plant^{-1})		Mean area per leaf (cm^2)	
			Stem	Branches	Stem	Branches
Clone AR3						
HW	152.3 ± 1.7 a	29 ± 1 a	27 ± 1 a	138 ± 7 a	41.46 ± 0.73 a	17.05 ± 0.63 a
MS	133.2 ± 2.4 b	20 ± 2 b	26 ± 2 a	68 ± 6 b	34.92 ± 2.29 b	12.26 ± 0.43 b
SS	113.3 ± 3.5 c	12 ± 1 c	23 ± 0 a	44 ± 5 c	33.43 ± 1.60 b	10.47 ± 0.56 b
Clone CN44						
HW	148.3 ± 5.1 a	29 ± 1 a	25 ± 0 a	140 ± 7 a	32.09 ± 0.81 a	16.61 ± 0.26 a
MS	118.5 ± 2.6 b	22 ± 2 b	24 ± 1 ab	94 ± 7 b	24.85 ± 1.95 b	10.91 ± 0.60 b
SS	98.0 ± 3.5 c	16 ± 1 c	22 ± 1 b	72 ± 3 b	22.86 ± 1.00 b	9.49 ± 0.28 b
Clone MP11						
HW	172.0 ± 2.1 a	24 ± 1 a	25 ± 1 a	103 ± 6 a	38.91 ± 1.97 a	19.30 ± 0.43 a
MS	141.4 ± 1.8 b	16 ± 1 b	22 ± 1 b	58 ± 4 b	33.70 ± 1.59 ab	11.94 ± 0.46 b
SS	117.3 ± 2.3 c	11 ± 0 c	20 ± 0 b	41 ± 2 c	31.64 ± 1.37 b	10.48 ± 0.51 b
Significance of two-way ANOVA						
Clone (C)	***	***	***	***	***	***
Watering regime (W)	***	***	***	***	***	***
C × W	ns	ns	ns	ns	ns	ns

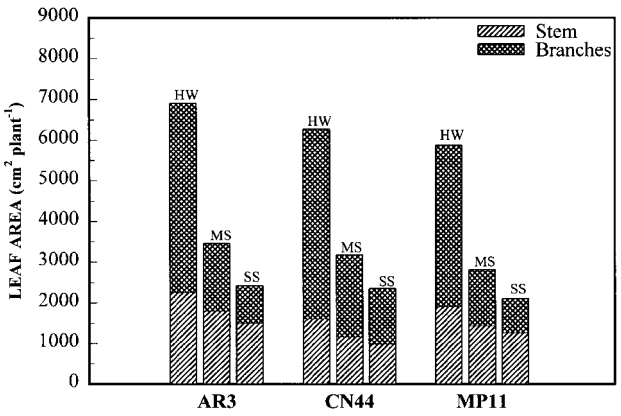

Figure 2. Effects of three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) on the leaf area produced on the main stem and on the branches by three *E. globulus* clones. Each value is the mean for six plants.

biomass allocated to branches and branch foliage in all clones, although the decrease was less marked in Clone CN44 than in the other clones. Conversely, the water-stress treatments increased the ratio of stem foliage biomass to total biomass in all clones. The proportion of dry matter allocated to roots was not affected by the watering regimes except in plants of Clone CN44, which had significantly lower root biomass in the MS and SS treatments than in the HW treatment. The watering regimes had no effect on the ratio of stem axis biomass to total biomass in Clone AR3. However, for Clones CN44 and MP11, significantly less biomass was allocated to the stem axis in plants in the SS treatments than in plants in the MS and HW treatments. In addition to differences in responses to water availability, the genotypes differed in biomass partitioning. Overall, Clone MP11 invested a larger proportion of total dry mass in the stem axis and Clone CN44 invested a larger proportion of total dry mass in branches and branch leaves than the other genotypes.

Compared with results of the conventional analysis presented in Table 2, allometric analysis provided a different picture of the effects of genotype and water availability on the allocation patterns of the *E. globulus* ramets (Table 3). When differences in plant size were adjusted statistically, the apparent effect of water deficits on dry matter partitioning among functionally distinct plant parts disappeared, indicating that water only indirectly affected allocation through accelerated growth. In contrast, genotype clearly affected most allometric coefficients between total plant dry weight and the dry weights of different plant components, indicating a direct effect of genotype on partitioning, thereby confirming that the genotypes differed in their biomass allocation patterns. The slopes of the regressions between total plant biomass and the biomass of different plant parts indicated that, in the AR3 and MP11 clones, dry matter was allocated preferentially to branches and leaves on branches, with roots and stems having low allometric coefficients. In contrast, in Clone CN44, partitioning to roots, stems and branches was favored at the expense of leaf production. Consequently, the allometric coefficient between foliage and root biomass was significantly higher in Clone CN44 than in the other two clones. The slopes of the regressions between total plant biomass and numbers of branches and leaf pairs on branches indicated an increased branching ability in Clone

Table 2. Variations in the ratios of root, stem, branch and leaf biomass to total dry matter with watering regime (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) in three *E. globulus* clones. Data are for the final harvest (Day 180). Each value is the mean for six plants ± standard error of the mean. For each clone and variable, a single factor analysis of variance was performed between water treatments. Means followed by the same letters are not significantly different at $P < 0.05$ (Bonferroni test). Asterisks *, **, *** represent significance at $P = 0.05$, 0.01 and 0.001, respectively; ns = nonsignificant at $P = 0.05$.

	Root:Total	Stem:Total	Branches:Total	Stem leaves:Total	Branch leaves:Total
Clone AR3					
HW	0.110 ± 0.005 a	0.289 ± 0.004 a	0.114 ± 0.003 a	0.167 ± 0.008 c	0.320 ± 0.004 a
MS	0.108 ± 0.006 a	0.290 ± 0.008 a	0.087 ± 0.008 b	0.277 ± 0.020 b	0.237 ± 0.014 b
SS	0.107 ± 0.004 a	0.289 ± 0.002 a	0.067 ± 0.004 c	0.340 ± 0.012 a	0.197 ± 0.010 c
Clone CN44					
HW	0.129 ± 0.006 a	0.284 ± 0.006 a	0.146 ± 0.004 a	0.126 ± 0.009 c	0.315 ± 0.006 a
MS	0.094 ± 0.005 b	0.262 ± 0.005 b	0.144 ± 0.006 a	0.185 ± 0.011 b	0.315 ± 0.013 a
SS	0.095 ± 0.004 b	0.255 ± 0.008 b	0.125 ± 0.001 b	0.227 ± 0.009 a	0.298 ± 0.006 a
Clone MP11					
HW	0.108 ± 0.009 a	0.332 ± 0.004 a	0.107 ± 0.003 a	0.157 ± 0.011 c	0.296 ± 0.007 a
MS	0.099 ± 0.003 a	0.314 ± 0.006 ab	0.089 ± 0.003 b	0.261 ± 0.011 b	0.238 ± 0.007 b
SS	0.097 ± 0.004 a	0.299 ± 0.005 b	0.072 ± 0.003 c	0.320 ± 0.010 a	0.213 ± 0.008 b
Significance of two-way ANOVA					
Clone (C)	ns	***	***	***	***
Watering regime (W)	***	***	***	***	***
C × W	*	*	*	*	***

Table 3. Effects of genotype and soil water availability on the allometric relationships $\log_e y = b_0 + b_1 \log_e x$ between selected pairs (x, y) of dimensions from *E. globulus* ramets. The F-values and significance levels of the ANCOVAs are presented for the effects of clone and water availability on the allometric coefficient b_1. Within a row, different letters indicate a significant difference ($P < 0.05$) between allometric coefficients b_1 of the clones, determined by the Tukey-Kramer test. Asterisks *, **, *** represent significance at $P = 0.05$, 0.01 and 0.001, respectively; ns = nonsignificant at $P = 0.05$.

$x - y$	Water availability	Clone	AR3	CN44	MP11
			b_1	b_1	b_1
$W_{total} - W_{root}$	0.697^{ns}	6.709^{**}	1.040 (0.053) b	1.341 (0.062) a	1.136 (0.062) b
$W_{total} - W_{stem}$	0.306^{ns}	4.049^{ns}	0.991 (0.022) a	1.084 (0.035) a	1.084 (0.022) a
$W_{total} - W_{branch}$	0.588^{ns}	11.610^{***}	1.494 (0.065) a	1.134 (0.040) b	1.356 (0.048) a
$W_{total} - W_{stem\ leaves}$	1.276^{ns}	0.856^{ns}	0.336 (0.053) a	0.410 (0.064) a	0.309 (0.047) a
$W_{total} - W_{branch\ leaves}$	0.464^{ns}	26.378^{***}	1.453 (0.042) a	1.058 (0.036) c	1.309 (0.038) b
W_{total} – NB[1]	1.100^{ns}	3.677^{*}	0.818 (0.090) a	0.501 (0.088) b	0.704 (0.070) ab
W_{total} – NLPB[2]	0.262^{ns}	10.687^{***}	1.079 (0.066) a	0.657 (0.059) c	0.868 (0.068) b
$W_{leaves} - W_{root}$	0.204^{ns}	7.466^{**}	1.134 (0.065) b	1.581 (0.095) a	1.323 (0.086) b
$W_{leaves} - W_{stem}$	0.160^{ns}	5.301^{**}	1.086 (0.025) b	1.275 (0.067) a	1.265 (0.047) a
$W_{leaves} - W_{branch}$	0.871^{ns}	3.957^{*}	1.627 (0.084) a	1.351 (0.051) b	1.583 (0.073) a
$W_{leaves} - W_{not\ leaves}$	0.147^{ns}	4.882^{*}	1.194 (0.026) b	1.360 (0.052) a	1.334 (0.046) a

[1] NB = Number of branches.
[2] NLPB = Number of leaf pairs on branches.

AR3 relative to the other two clones, which was confirmed by the finding that the allometric coefficient between the biomass of the assimilatory and the non-assimilatory plant parts ($W_{assim} - W_{non-assim}$) was lower in Clone AR3 than in the other clones.

Leaf growth

The time course of leaf lamina expansion between Days 119 and 160 illustrates the effects of the watering regimes on the growth of individual leaves (Figure 3). Soil water deficits reduced final leaf surface areas and maximum rates of leaf area expansion. The results obtained by fitting the Richards curves (Table 4) demonstrated that final leaf area, maximum relative expansion rate and duration of leaf growth were altered by soil water deficits. Overall, final leaf size in plants grown in the MS and SS treatments was 80 and 50%, respectively, of that of HW plants. Well-watered plants had a maximum relative expansion rate that was 1.5 times as great as that of MS-treated plants and

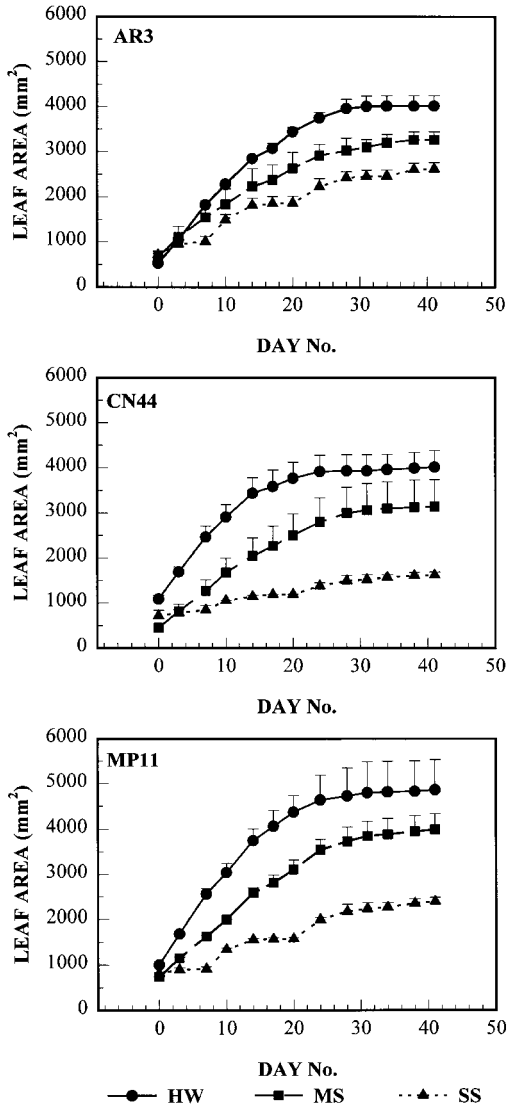

Figure 3. Effects of three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) on the time course (Days 119 to 160) of leaf area expansion in stem leaves of three *E. globulus* clones. Mean ± standard error of measures for three plants per treatment.

3 times as great as that of SS-treated plants. Water limitation resulted in a 12-day increase in the duration of leaf growth in MS-treated plants and a 20-day increase in SS-treated plants. The SS treatment had smaller effects on final leaf size and maximum relative growth rate in Clone AR3 than in the other two clones. Overall, clones differed significantly in final leaf size (MP11 > CN44, with AR3 between the two), maximum relative growth rate of area expansion (AR3 > MP11, with CN44 between the two) and duration of leaf growth (AR3 = MP11 > CN44). The shape of the leaf expansion curves was fairly smooth for plants in the HW and MS regimes (Figure 3). In contrast, the pattern observed in SS-treated plants showed discontinuity, with a steep resumption of leaf expansion following rewatering, especially in Clones AR3 and MP11.

Table 4. Effects of three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) on growth of stem leaves in three *E. globulus* clones. Parameters derived from the Richards curves were fitted to untransformed leaf area data: S_x = final value of leaf area; r = relative growth rate at the point of inflexion; $t_{5\%-95\%}$ = duration between 5 and 95% of final growth. Each value is the mean for three plants ± standard error of the mean. For each clone and variable, a single factor analysis of variance was performed between water treatments. Means followed by the same letters are not significantly different at $P < 0.05$ (Bonferroni test). Asterisks *, **, *** represent significance at $P = 0.05$, 0.01 and 0.001, respectively; ns = nonsignificant at $P = 0.05$.

	S_x (cm^2)	r (day^{-1})	$t_{5\%-95\%}$ (days)
Clone AR3			
HW	41.60 ± 1.41 a	0.13 ± 0.01 a	34.1 ± 1.0 a
MS	33.80 ± 0.35 b	0.09 ± 0.01 b	48.3 ± 1.5 b
SS	27.20 ± 0.63 c	0.06 ± 0.01 c	54.1 ± 1.8 c
Clone CN44			
HW	40.00 ± 2.01 a	0.12 ± 0.00 a	28.8 ± 0.5 a
MS	32.80 ± 3.62 a	0.10 ± 0.00 b	40.8 ± 0.2 b
SS	17.70 ± 0.37 b	0.03 ± 0.01 c	46.7 ± 0.9 c
Clone MP11			
HW	49.40 ± 4.40 a	0.12 ± 0.01 a	32.3 ± 1.9 a
MS	41.20 ± 1.96 a	0.07 ± 0.00 b	44.0 ± 0.7 b
SS	24.80 ± 0.08 b	0.04 ± 0.00 c	54.9 ± 1.7 c
Significance of two-way ANOVA			
Clone (C)	***	*	***
Watering regime (W)	***	***	***
C × W	ns	ns	ns

The time course of leaf elongation rate (LER) showed that, in SS-treated plants of all clones, leaf expansion had a rhythmicity that was paralleled by soil water availability (Figure 4). In response to the dehydration–rewatering cycles, oscillations of decreasing amplitude were observed, with LER rising from a near zero value on the day before rewatering to a peak value around 2 days after water stress alleviation. The amplitude of changes in LER was greater in Clones AR3 and MP11 than in Clone CN44 (Figure 3). The irreversibility of leaf dimensions after rewatering (Figure 3) demonstrates that recovery cycles are not an artifact created by shrinkage–rehydration processes.

The patterns of individual leaf growth may be related to changes in leaf water relations during the intervals between two watering events (Figures 5 and 6). Relative water content (RWC) remained roughly uniform throughout the period between two watering events in the HW- and MS-treated plants, but was significantly lower than the initial value by the middle of the interval in SS-treated plants (Figure 5). In contrast, predawn leaf water potential (Ψ_w) decreased significantly by the end of the interval between watering events in plants in all of the treatments, with much lower values in the SS-treated plants than in the HW- and MS-treated plants (Figure 5).

Leaf turgor potentials (Ψ_P) decreased significantly during the interval between watering events in all plants except the SS-treated plants of Clone AR3 (Figure 6). Osmotic potentials

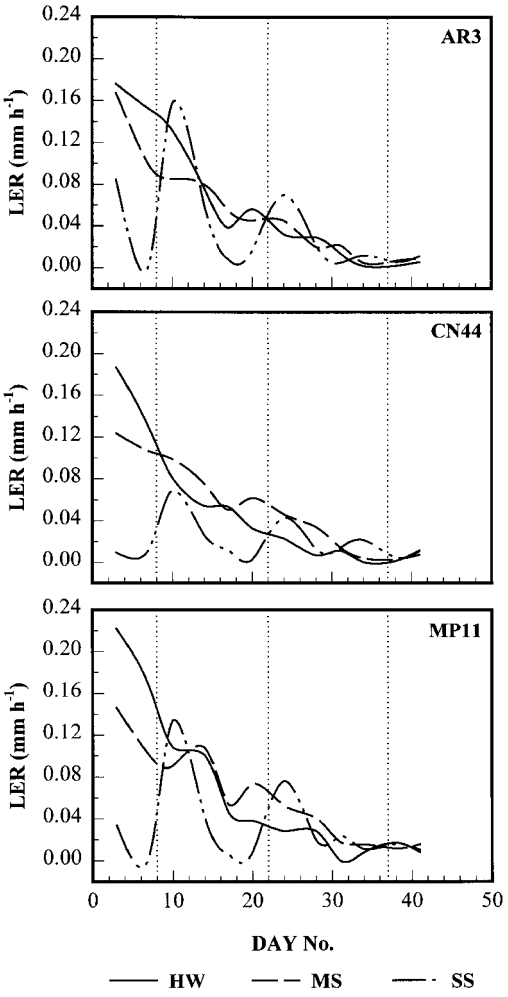

Figure 4. Effects of three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) on the leaf expansion rate (LER) of stem leaves of three *E. globulus* clones. See Figure 2 for details. For each watering regime, a smooth curve passing by the mean points was drawn applying a cubic spline interpolation. Vertical dotted lines indicate the days when severely water-stressed plants were rewatered.

at full turgor (Ψ_π^{100}) immediately after watering and during the period between irrigation events were not significantly different in the study plants except for the SS-treated plants. In SS-treated plants of Clone AR3, Ψ_π^{100} decreased substantially throughout the interval between watering events; however, leaf turgor potential (Ψ_P) did not change significantly during the same period, indicating that osmotic adjustment occurred in this clone. Conversely, the reductions in Ψ_π^{100} in SS-treated plants of the CN44 and MP11 genotypes were not sufficient to preserve leaf turgor between two watering events (Figure 6).

Discussion

As reported for many other woody species (e.g., Wang et al. 1988, Myers and Landsberg 1989, Ranney et al. 1990, Steinberg et al. 1990, Rhodenbaugh and Pallardy 1993), soil water deficits affected biomass accumulation and growth of young *Eucalyptus* plants. Results from field and controlled environment experiments with several tree crops suggest a linear association between total dry matter production and total solar radiation intercepted by the leaves during the growing season (Byrne et al. 1986, Cannell 1989, Pereira 1990). By the end of the 180-day experiment with the three *Eucalyptus* clones, variation in total leaf area accounted for approximately 94% of the variance in total dry matter, and water deficits reduced total biomass in proportion with plant foliage. Therefore, we conclude that the long-term reduction in biomass accumulation in water-stressed plants was a result of decreased foliage area and intercepted solar radiation. However, it is possible that, in addition to decreases in foliage area, water deficits resulted in lower carbon assimilation rates per unit leaf area in the short term. The latter effect was probably related to the length of time during which the plants had open stomata (cf. Osório and Pereira 1994), because drought-induced reductions in stomatal conductance (g_s) paralleled the drought-induced reductions in total biomass (a 50% reduction in g_s in MS-treated plants and a 60% reduction g_s in SS-treated plants, unpublished observations).

Small volumes of rooting medium may restrict plant growth (e.g., Ismail et al. 1994). However, we used relatively large pots (10 liters) in relation to plant size and at the time of harvest there was no visible indication that root growth had been restricted, indicating that pot size had no specific effects on the patterns of root and shoot growth and relative allocation.

Water-stressed plants of *E. globulus* released fewer lateral branches from apical dominance and allocated less biomass to branches than well-watered plants. Similar results have been observed in the field (Pereira 1990). Because branch leaves accounted for as much as 70% of whole-plant foliage in the well-watered plants, we conclude that reductions in lateral branching and new leaf production on branches were primarily responsible for the decreases in light interception and biomass production caused by water shortage. Moreover, the reductions in whole-plant leaf area resulted from water stress effects on foliage growth rather than leaf shedding, which was negligible in all treatments.

Even though a reduction in leaf numbers on branches accounted for most of the water-stress-induced decrease in whole-plant foliage area, decreased leaf expansion also contributed substantially to the reduction (cf. Table 1). Thus, parameters of the Richards equation for lamina growth were strongly affected by water deficiency, with leaves on the stem axis of water-stressed plants exhibiting reduced final size, decreased rates of expansion and about a 1.5-fold increase in the time needed to reach final leaf size. Effects of limited water availability on area development of branch leaves may be more pronounced than that on leaves of the stem axis because of increased hydraulic resistance to water flow in branches (Yang and Tyree 1993, Mencuccini and Grace 1996). The reduction in mean leaf size caused by water stress on branches was about twice that on the stem (Table 1).

The leaves of SS-treated *E. globulus* plants responded rapidly (2 days) to rewatering by resuming growth, but contrary to the observations of Metcalf et al. (1990), they did not attain

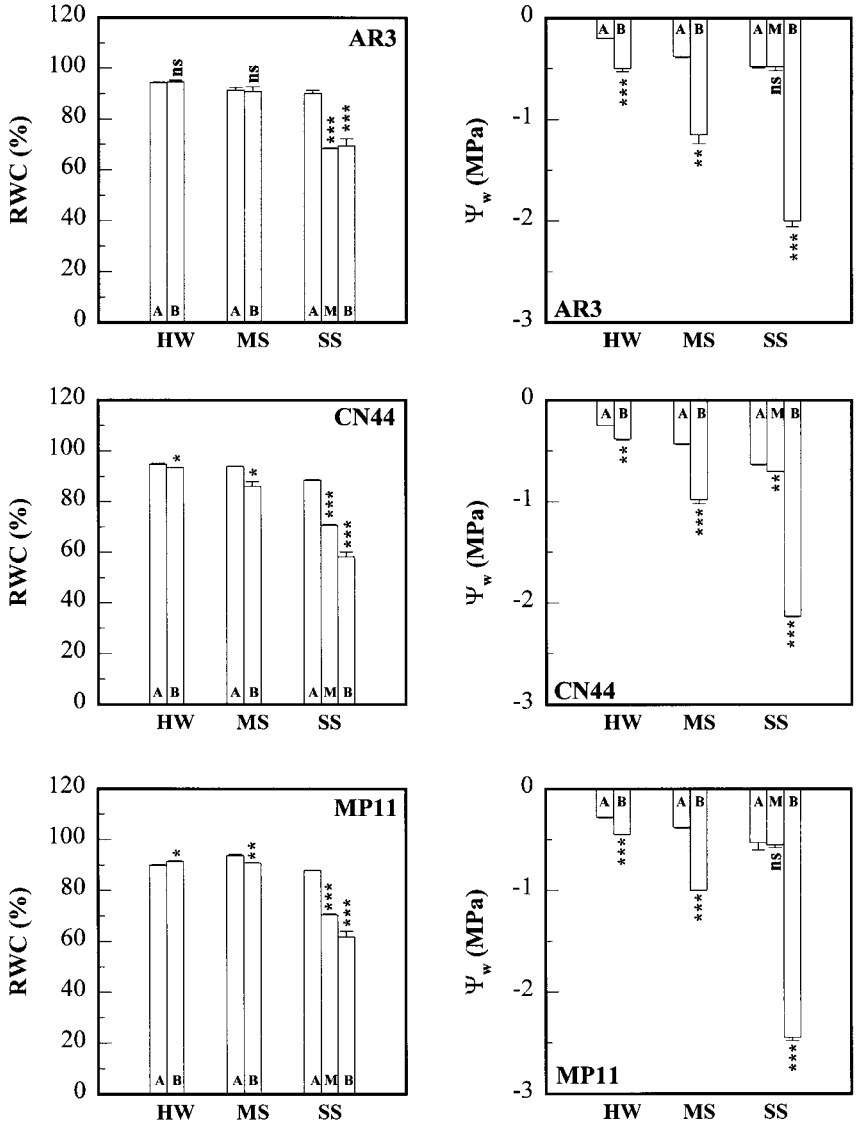

Figure 5. Variations in the predawn values of relative water content (RWC) and water potential (Ψ_w) of stem leaves of three *E. globulus* clones subjected to three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) during the time interval between two watering operations. A = 24 h after last irrigation, M = at the middle of the watering interval and B = 24 h before next irrigation. Mean ± standard error of measures for four plants per treatment. Values measured at M and B were compared with those measured at A by the Dunnett's test. Asterisks *, **, *** represent significance at $P = 0.05$, 0.01 and 0.001, respectively; ns = nonsignificant at $P = 0.05$.

the same final size as leaves on plants that had been continuously well watered. The ability to resume leaf expansion following relief of water stress has been demonstrated in several crop species (e.g., Rawson and Turner 1982, Palta 1984) and may be particularly important for early seedling establishment and productivity in drought-prone environments.

The genotypes differed in rate and degree of leaf area expansion once water stress was alleviated. Leaves of Clone AR3 attained greater expansion rates after rewatering than leaves of the other genotypes (Figure 4), and this recovery appeared to be associated with an enhanced capacity for active osmotic adjustment in response to severe soil water deficits. In contrast with Clones CN44 and MP11, leaf turgor of SS-treated plants of Clone AR3 was maintained throughout the period between two watering events concomitantly with a substantial decrease in Ψ_π^{100} (about 0.7 MPa, Figure 6). Some studies suggested the involvement of osmotic adjustment in the response of eucalypts to soil water deficits (Myers and Neales 1986, Bachelard 1986*a*, Correia et al. 1989, Stoneman et al. 1994). However, we believe that stomatal functioning is one of the key factors in the acclimation of *E. globulus* to water shortage (see also Osório and Pereira 1994, Pereira and Osório 1995). A similar conclusion was reached by Edwards and Dixon (1995) for eastern white cedar (*Thuja occidentalis* L.).

It has been argued (e.g., Coleman et al. 1993, Gebauer et al. 1996) that the interpretation of changes in allocation patterns in response to resource supply may be misleading if differences in plant size are not taken into account. The results obtained in the present study add credence to this viewpoint. An effect of soil water availability on biomass partitioning was found when data were analyzed in the traditional fashion; however, when an allometric analysis was carried out to remove the effect of large differences in size among plants in the different treatments, the apparent effects of the watering regimes on biomass allocation disappeared, but large differences in patterns of biomass allocation among the genotypes were revealed. Clones AR3 and MP11 preferentially allocated dry matter to leaves on branches, whereas Clone CN44 preferen-

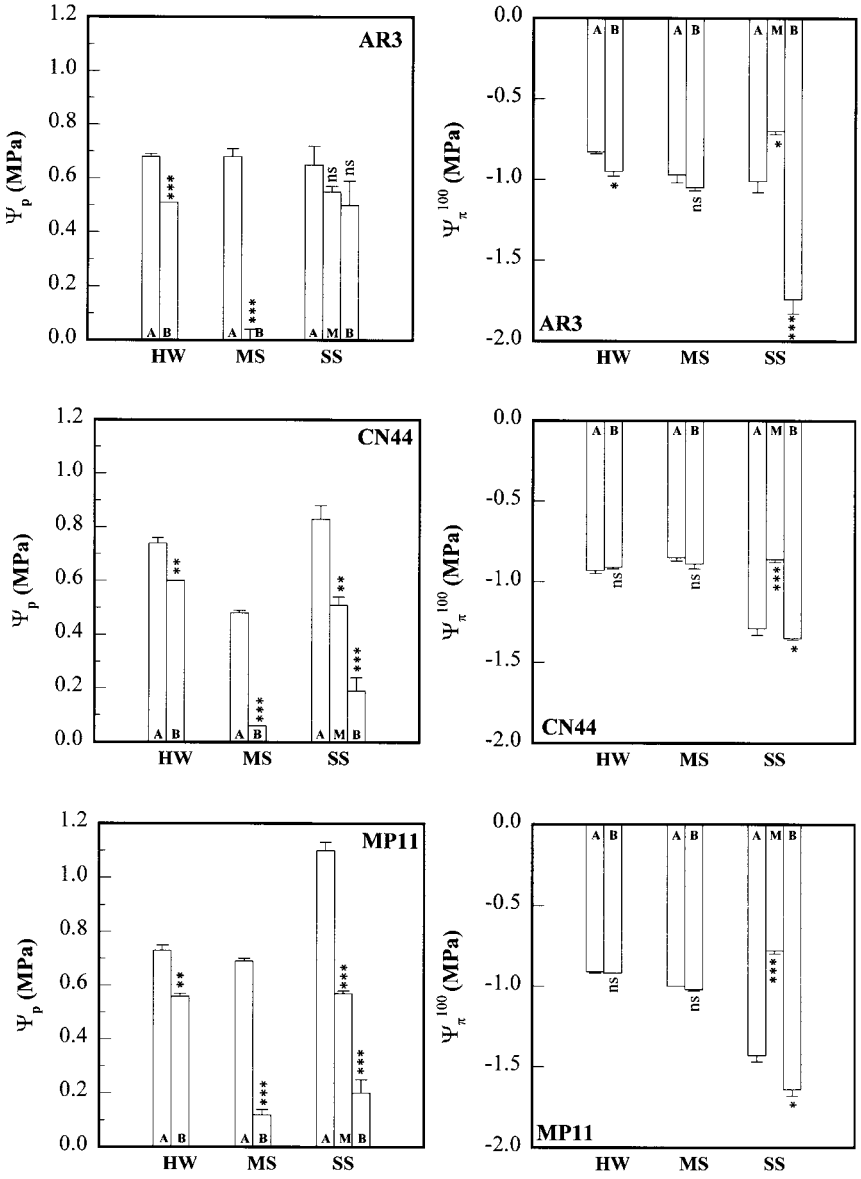

Figure 6. Variations in the predawn values of the turgor potential (Ψ_P) and osmotic potential at full turgor (Ψ_π^{100}) of stem leaves of three *E. globulus* clones subjected to three watering regimes (HW = well watered, MS = moderate soil water deficit and SS = severe soil water deficit) during the time interval between two watering operations. See Figure 4 for details. Asterisks *, **, *** represent significance at P = 0.05, 0.01 and 0.001, respectively; ns = nonsignificant at P = 0.05.

tially allocated dry matter to non-assimilatory organs (Table 3). These genotypic differences in the pattern of biomass allocation in turn affected the response of clones to soil water deficits. For instance, Clone CN44, in which the allometric coefficient between leaves and root biomass ($W_{leaves} - W_{root}$) was significantly higher than in the other two genotypes, exhibited a lower sensitivity of lateral branching, leaf initiation and whole-plant foliage area to water stress than the other two clones.

An increase in root to shoot ratio (or root to total dry matter ratio), attributable mainly to a reduction in shoot growth, has often been observed when water is limiting (e.g., Sharp and Davies 1979, Bachelard 1986*b*, Steinberg et al. 1990). However, none of the clones studied here exhibited an increase in root:shoot biomass ratio in response to a shortage of soil water. Similar findings have been reported for both pot-grown (Pereira and Kozlowski 1976) and field-grown (Pereira et al. 1987) *E. globulus*. Thus, our data are consistent with the hypothesis that *E. globulus* plants do not exhibit an "optimizing strategy" for root growth whereby more biomass is allocated to roots than is needed to maintain maximal relative growth rates under drought conditions (Pereira and Pallardy 1989). Similarly, Farrell et al. (1996) did not find significant differences between the root:shoot ratios under drought cycles compared to the ratios observed under conditions of half-waterlogging in potted plants of clonal lines of *Eucalyptus camaldulensis* Dehnh., a species with greater tolerance to many environmental stresses than *E. globulus*. Based on these data, we conclude that genetic control over the root:shoot ratio is very strong in some *Eucalyptus* species.

Separation of the size-dependent changes in biomass partitioning from direct changes in the carbon allocation process revealed that water deficits probably act on biomass allometry by slowing down growth and adjusting plant size to the re-

duced amounts of carbon assimilated as a result of stomatal closure and decreased leaf area (determined by previous stress events). We speculate that direct (short-term) effects such as reduced net photosynthesis and inhibition of leaf expansion after each water stress episode led to less fixed carbon and increased apical dominance, resulting in a lower growth potential because of reductions in foliage area and hence the amount of solar radiation intercepted by the crown.

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