SILVA FENNICA

Silva Fennica 41(2) research articles

www.metla.fi/silvafennica · ISSN 0037-5330
The Finnish Society of Forest Science · The Finnish Forest Research Institute

Interactions between Morphological and Physiological Drought Responses in *Eucalyptus microtheca*

Sanna Susiluoto and Frank Berninger

Susiluoto, S. & Berninger, F. 2007. Interactions between morphological and physiological drought responses in *Eucalyptus microtheca*. Silva Fennica 41(2): 221–233.

We studied the response of *Eucalyptus microtheca* to drought in a greenhouse experiment. As a result of the drought the growth of the seedlings decreased and allocation patterns changed so that allocation to the roots increased. However, changes in photosynthesis and stomatal conductance under drought were rather modest. We showed, using chlorophyll fluorescence and measurements of photosynthesis under high CO_2 that the biochemical capacity of photosynthesis increased under drought. The results suggest that changes in root/shoot ratio are the primary reactions that initiate a series of compensatory reactions that mitigate the effects of drought in *Eucalyptus microtheca*.

Keywords chlorophyll fluorescence, photosynthesis, root/shoot ratio, RuBP, xylem permeability
Addresses *Susiluoto*: University of Helsinki, Department of Forest Ecology, PL 27, 00014 University of Helsinki, Finland; *Berninger*: Departement des Sciences biologiques, Université du Québec à Montréal, CP 8888 Succ Centre Ville, Montreal, Canada
E-mail sannamaija.susiluoto@helsinki.fi
Received 18 November 2005 **Revised** 19 January 2007 **Accepted** 8 February 2007
Available at http://www.metla.fi/silvafennica/full/sf41/sf412221.pdf

1 Introduction

Discussion on the effects of drought has largely focused on either physiological or morphological acclimations to drought stress that make plants more resistant and more productive. The interplay between morphological and physiological factors is not well understood and deserves more attention. For example, increases in the root/shoot ratio as observed in many studies (e.g. Bachelard 1986; Li and Wang 2003) will bring along changes in the nutrition of plants, and these will in turn affect the photosynthetic system.

There is an ongoing debate on the effects of drought on the physiology of photosynthesis. Farquhar et al. (2002) claim, based on theoretical arguments, that under water limitations plants allocate more nitrogen to Rubisco. On the other hand, Tezara et al. (1999) show that drought stress leads to an inhibition of ribulose bisphosphate

synthesis, which is related to a loss of ATP regeneration. Kaiser (1987) found in a review study that moderate drought stress has little effect on photosynthetic capacity. Though, in some Australian tree species photosynthesis, even under conditions of nitrogen deficiency, is not limited by Rubisco activity, but by the decreased absorption of light (Warren et al. 2000). Many of these species also tend to have Rubisco in excess compared to its need for photosynthesis in optimal conditions and Warren et al. (2000) concludes that the high concentrations of Rubisco may defend plants against photoinhibition: High Rubisco activities help plants to maintain higher rates of photosynthesis, i.e. use a substantial part of the light energy for photosynthesis under partial stomatal closure, and make it less necessary to use photoinhibition to protect themselves against high light (Warren et al. 2000). Often, both stomatal and nonstomatal properties of photosynthesis decline together, even though the major part of the reduction on photosynthesis can be accounted by stomatal effects (Collatz et al. 1976).

Usually during periodical drought stress leaves close their stomata, which in turn decreases the substomatal CO_2 concentration as the diffusion of CO_2 into the leaves is restricted (Chaves 1991). This decreases the net photosynthesis rate. Many plants decrease their RuBP content and impair ATP synthesis as an early response to drought. Decreased Rubisco activity and photoinhibition occur many times in later phases of drought (Flexas and Medrano (2002), even though these metabolic phases follow the initial reaction of stomatal closure (Flexas and Medrano 2002; Bota et al. 2004).

When substomatal CO_2 concentrations decrease the rate of dark reactions of photosynthesis also decrease, since the supply of CO_2 to the Calvin cycle is reduced. Light reactions, on the other hand, will go on at full speed and plants have to divert the excess energy from the photosystems into harmless products. Photoinhibition, the closure of photosystem II reaction centers and the conversion of light into harmless heat are such a protection mechanisms (Maxwell and Johnson 2000). Photoinhibition does not occur always, but depends on the species and the severity of the drought (Damesin and Rambal 1995; Sánchez-Rodriguez et al. 1997; Tambussi et al. 2002).

The natural habitats of *Eucalyptus microtheca* (F. Muell.) cover a large area in mid-northern and northern parts of Australia in arid and semi-arid growth areas. It grows mostly in open woodlands in between 14°–33°S latitude and up to 700 m in altitude range. *E. microtheca* grows on very different soils, even though heavy or brown, self-mulching and cracking clays predominate (CSIRO 1979). *E. microtheca* is the *Eucalyptus* species which is frequently planted in the driest habitats for industrial and non-industrial purposes (Tuomela 1997). It has been successfully cultivated in Sudan, Iran, Iraq and Pakistan (FAO 1979). Tuomela (1997) showed that eastern populations of *E. microtheca* have a prodigal water use strategy and a faster growth rate than western populations. Eastern Australia has, compared to Western Australia, a more evenly distributed rainfall, while Western Australia has a well defined predictable dry season. Li et al. (2000) showed, furthermore, that the severeness of the dry season at their origin determines largely the responses of *E. microtheca* to drought.

The aim of this research was to study the physiological and morphological changes in seedlings of *E. microtheca* from Western Australia during drought. The hypotheses were that during the dry period the seedlings of *E. microtheca* would transpire less, grow less, increase their root/foliage ratio and photosynthesize less than under normal conditions.

2 Material and Methods

Eucalyptus microtheca from Longreach, 23°26´S and 144°16′E (seedlot 12494, Australian Tree Seed Centre, CSIRO Forestry and Forest Products, Canberra) was used in this study. The provenance originates from low open woodlands on loamy duplex soils and forms extensive stands on flood plains and margins of rivers and creeks. The climate in the area is hot and semi-arid with a winter dry season. The mean annual precipitation is 443 mm (records available from 72 years). The mean annual maximum temperature is 31.3°C and the mean annual minimum temperature 15.5°C (CSIRO 1979).

Seed germination was started on October 1st, 2001 on wet tissue paper at 20–25°C. Seedlings were planted into 0.18 liter pots, which were filled with a mixture of sand and complete fertilizer (size 0.5–1.2 mm), and moved into the greenhouse on the 19th Oct., 2001. The greenhouse (located in Helsinki, Finland) temperature was set at +25°C during daytime and +20°C during nights. The relative humidity was set to 70% RH around the clock. The photoperiod was 12 hours/day and light was supplied by lamps with a photon flux density of 500–700 μmol $m^{-2} s^{-1}$.

Seedlings were replanted into 2 liter pots filled with 2.5 kg of sand (grain size 0.5–1.2 mm) on 15th and 16th Dec. 2001. Before the start of the drought treatment the seedlings were watered every second or third day to field capacity. Seedlings were fertilized about once a week by using "Gardeners general fertilizer" ("Puutarhan yleislannos", Kemira Ltd, Helsinki, Finland), which has N-P-K-ratio of 8-4-14. The amount of fertilizer given to plants was calculated according to the estimated mean leaf area of the average seedlings (300 g of fertilizer/1 m^2 of leaf area/seedling).

The drought phase of the experiment started when seedlings were 3.5 months old (15th Jan., 2002). Forty-five healthy and vigorous seedlings were randomly assigned to three irrigation treatments, which were irrigated to 15, 30 and 100% of field capacity. For shortness these treatments will be called 15%, 30% and control, respectively. All seedlings reached these values of soil water content on 25th Jan. 2002. After that the seedlings were weighed and watered daily at 10–11 am. To avoid any systematic error due to possible differences in growth conditions in the greenhouse, the seedlings were circulated about once a week after the start of the drought experiment. The measurement period ended at 22nd Feb. 2002 when seedlings were about 4.5 months old.

2.1 Growth and Morphology of the Seedlings

At the end of the experiment on 23rd Feb.–5th March, the dry weights (dried about 60 hours at +55°C) were measured from the leaves, stem, fine roots (diameter < 2 mm) and coarse roots (> 2 mm) of each seedling. Dry weights and leaf areas of eight randomly selected leaves from each seedling were measured with a Li-cor leaf area meter (LI-3050A, Li-cor, Lincoln, USA).

2.2 Physiological Determinations

Transpiration was measured gravimetrically from all seedlings (15 in each treatment) every day between 10–11 am from 18th Jan. 2002 to 22nd Feb. 2002. To minimize errors due to evaporation from the soil surface, the pots were kept in waterproof plastic bags, which were tied up loosely around the stems. Evaporation from the soil surface was estimated from pots without seedlings (but wooden sticks to simulate the evaporation between stem and plastic bag). Transpiration values in this paper have been corrected for evaporation from the soil.

Measurements of photosynthesis were made by CIRAS2 portable photosynthesis measurement system (PP Systems, Hitchin and Hertfordshire – UK) at +25°C during late February. Six seedlings were measured from treatments Control and 30%, five from treatment 15%. Five repetitions from every measurement were taken. Photosynthesis and stomatal conductance were measured at high light (1.5 mmol $m^{-2} s^{-1}$) at both ambient (360 ppm) and elevated carbon dioxide concentrations (1500 ppm) at relative humidity (RH) of 63%. Water use efficiency was calculated by dividing photosynthesis with transpiration using the measurements done in ambient CO_2 concentration and 63% relative humidity.

Chlorophyll fluorescence was measured with a Hansatech Fluorescence Monitoring System (FMS) at a temperature of +25°C on 14th to16th Feb. 2002 for 10 seedlings from each treatment. The part of the leaf, which the measurement was made from, was kept in darkness for a period of 30 minutes before the measurement started. Terminology and calculations of fluorescence followed Maxwell and Johnson (2000). The variables measured were the minimal fluorescence yield (F_0) the maximum variable fluorescence emission (F_M), the steady state fluorescence (F_S) and the light adapted fluorescence maximum (F_M'). F_0 was determined by activation of the measuring beam (< 1μmol $m^{-2} s^{-1}$ at 1.6 KHz), and then a saturating flash (1s) was activated.

F_V/F_M describing the potential maximal quantum efficiency of PSII was calculated by the following formula: $F_V/F_M = (F_M - F_0)/F_M$. Also qP measuring photochemical quenching and NPQ measuring non-photochemical quenching were calculated by the following formulas: $qP = (F_M' - F_S)/(F_M' - F_0)$ and $NPQ = (F_M - F_M')/F_M'$.

The permeability of xylem was determined by measuring the rate at which water passes through a cut section of stem, with a known cross sectional area, under a steady pressure. The stem segments were taken about 2 cm above ground. The measurement was done at room temperature (+21°C) for nine seedlings from control and 15% treatments and for ten seedlings from 30% treatment at the end of the experiment on 3rd to 5th March, 2002. The permeability was determined as rapidly as possible after cutting the stem under water. Typical pressure differences were about 1 kPa for a piece of wood 2.1–4.4 cm long and 2.81–4.14 mm in diameter. Six measurements from every piece of stem were taken with five minutes interval. From the treatment 15% two of the measurements were deleted as outliers because of the results that deviated significantly from the others. We argue that this was due to a leak between the piece of wood and the pipe. Permeability (m^2) was calculated using Darcy's law according to Whitehead et al. (1984). Results of conductivity are calculated for each seedling as a mean value of the six measurements made during the period of five minutes.

Nitrogen samples were analyzed by LECO 1100 elemental analyzer (LECO Corporation, St. Joseph, MI, USA) from 6 seedlings of each treatment.

Using the theoretical arguments of Long and Bernacchi (2003), the ratio of photosynthesis and intercellular CO_2 concentration (A/ci) in high light and ambient CO_2 was used to estimate the Rubisco activity. According these authors, the slope of these two values estimates the limitation of photosynthesis set by Rubisco activity. According to same theory, the photosynthesis level on a saturated intracellular CO_2 concentration describes the RuBP regeneration capacity.

The mean values and the standard errors of the mean were calculated for all variables, and differences between treatments tested by analysis of variance (ANOVA) using Tukey test. Pearson correlation coefficients were used to test correlations between measured variables. Statistical analyses were done with SYSTAT 6.0 statistical software package.

3 Results

The total biomass in the drought treatments was lower than in the control treatment and the differences in biomass between all three groups were statistically significant at $p < 0.05$ (Table 1). The stressed seedlings had higher root/shoot ratio (results were statistically significant at $p < 0.05$ between the control treatment and the drought stressed treatments but the stressed treatments did not differ statistically from each other) than the seedlings belonging to the control group and they grew more fine roots compared to the leaf area, even though the results in fine roots /leaf area did not differ statistically ($p > 0.05$, Table 1). Leaf area decreased and the specific leaf area (SLA) increased under drought stress (both results were statistically significant between the control treatment and the stressed treatments but the stressed treatments did not differ statistically from each other, Table 1). The ratio of coarse and fine root mass decreased with drought stress and this difference was significant ($p < 0.05$) between the control group and 15% treatment group (Table 1).

Transpiration per seedling increased with time in the control treatment, while in the 15% treatment it decreased and in 30% treatment it increased during the 10 first days of experiment. After this the transpiration in both stressed groups remained relatively constant (Fig. 1). Differences in transpiration between treatments were statistically significant ($p < 0.05$) starting from the eighth day after irrigation was reduced. Transpiration was also reduced by drought when the results were expressed per unit of leaf area (Table 1).

Xylem permeability was stable over time during the 30-minute period used in the study. Conductivity of xylem did not change due to drought ($p > 0.05$), neither on a xylem area basis nor expressed as leaf specific conductivity (Fig. 2).

There were no statistically significant differences in the net photosynthesis of seedlings

Table 1. Mean values and standard errors of water use efficiency (WUE, mol m^{-2} s^{-1}) leaf area (LA, cm^2), root/shoot ratio, coarse root/fine root ratio, specific leaf area (SLA, dm^2 g^{-1}), daily transpiration per leaf area (Trans / LA, g H_2O d^{-1} m^{-2}), fine roots / leaf area ratio (roots < 0.2 cm, g cm^{-2}), nitrogen concentration of leaves / leaf area (Nitrogen / LA, mg g^{-1} cm^{-2}), nitrogen acquisition by fine roots (leaf N × leaf mass / fine root mass, mg g^{-1}) and total biomass (g) in different treatments. Mean values marked with different letters differ statistically from each other at $p < 0.05$ according to Tukey's test. Note that the amount of fertilizer was given to plants according to the estimated mean leaf area of the average seedling. Number of seedlings in each treatment was 14 for control group and 15 for 15% and 30% treatments in all the other calculations except for nitrogen / LA, where number of seedlings was 6 in each treatment.

Treatment	15%	30%	Control
WUE	9.90 ± 1.40 (A)	7.29 ± 0.72 (A,B)	4.28 ± 0.28 (B)
LA	510.29 ± 26.07 (A)	646.96 ± 26.69 (A)	794.05 ± 64.15 (B)
Root / Shoot ratio	0.45 ± 0.02 (A)	0.45 ± 0.03 (A)	0.35 ± 0.02 (B)
Coarse / fine root ratio	0.88 ± 0.06 (A)	1.07 ± 0.09 (A,B)	1.25 ± 0.11 (B)
SLA	0.91 ± 0.03 (A)	0.80 ± 0.05 (A)	0.64 ± 0.04 (B)
Transpiration / LA	0.06 ± 0.004 (A)	0.12 ± 0.007 (B)	0.19 ± 0.014 (C)
Fine roots / LA	41.56 ± 3.05 (A)	40.66 ± 3.41 (A)	35.35 ± 3.35 (A)
Nitrogen / LA	0.03 ± 0.003 (A)	0.02 ± 0.002 (B)	0.01 ± 0.001 (C)
leaf N × leaf mass /fineroot mass	20.84 ± 1.97 (A)	17.51 ± 1.94 (A)	9.28 ± 1.38 (B)
Total biomass	12.24 ± 0.45 (A)	17.13 ± 0.70 (B)	22.88 ± 1.25 (C)

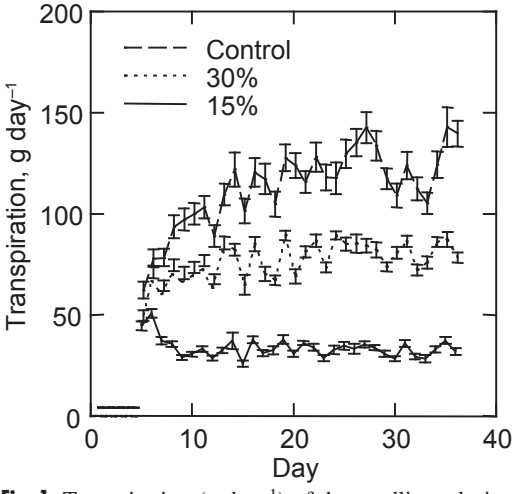

Fig. 1. Transpiration (g day^{-1}) of the seedlings during the drying experiment (18th Jan.–22nd Feb., 2002). Lines represent the standard errors of mean. The daily transpiration values of treatments differed statistically from each other at $p < 0.05$ (Tukey's test) from the eighth day forward.

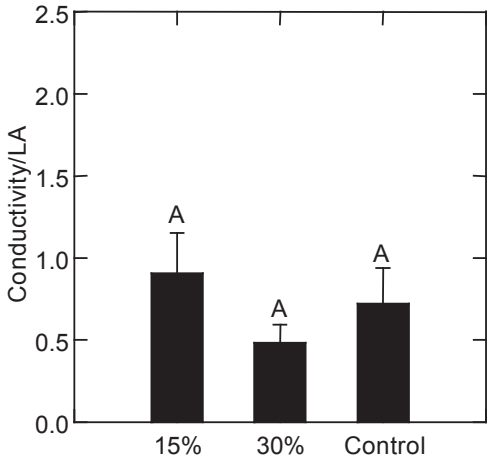

Fig. 2. Conductivity of xylem (10^{-9}). Lines represent the standard error of the mean. There were no statistically significant differences between treatments at $p < 0.05$, Tukey's test.

between treatments when photosynthesis was measured at ambient CO_2 concentrations (Fig. 3a). Mean stomatal conductance of the seedlings in 15% treatment was lower than the mean stomatal conductance in the other two groups, but the means did not differ statistically (Fig. 4b). However, the water use efficiency was higher ($p < 0.05$) in stressed groups than in control group

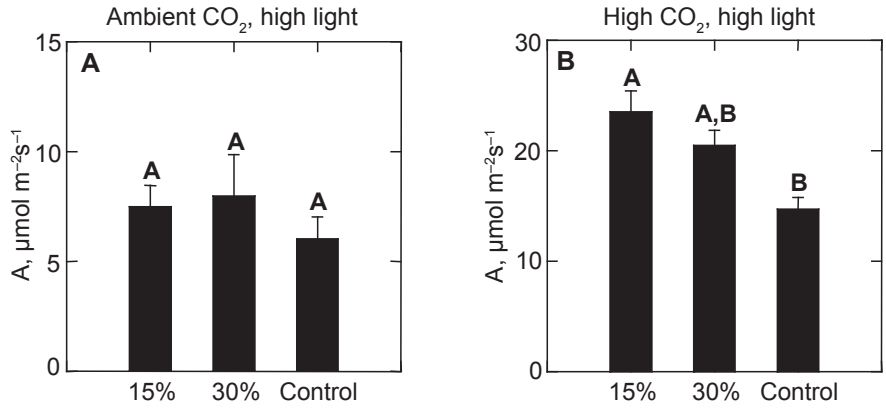

Fig. 3. A) Net photosynthesis rate (µmol m^{-2} s^{-1}, at PPFD 1500, µmol m^{-2} s^{-1}, RH 63% and CO_2 concentration of 360 ppm) and B) the rate of photosynthesis (µmol m^{-2} s^{-1}) in elevated CO_2 (1500 ppm) and high light intensity (PPFD 1500, µmol m^{-2} s^{-1}). Lines in the bars represent the standard errors of mean. Means marked with different letters differ statistically from each other at $p < 0.05$, Tukey's test.

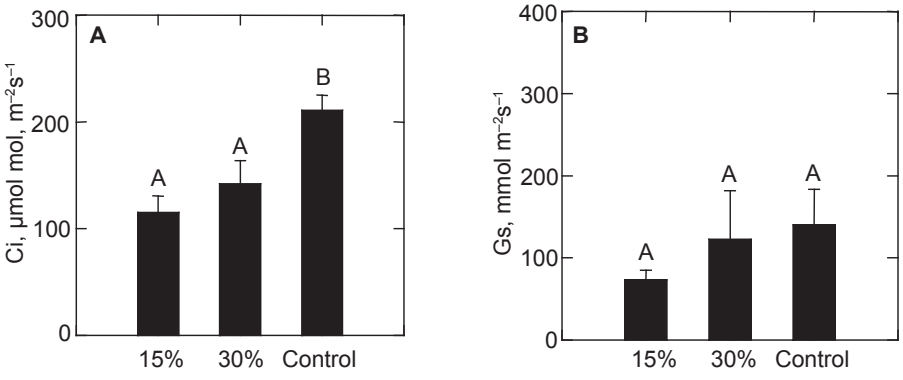

Fig. 4. A) Intercellular CO_2 concentration (Ci, figure a) and B) stomatal conductance (mmol m^{-2} s^{-1}), when RH = 63%, CO_2 = 360 ppm and PPFD = 1500 (µmol m^{-2} s^{-1}). Lines in the bars represent the standard errors of mean. Means marked with different letters differ statistically from each other at $p < 0.05$, Tukey's test.

(Table 1). Also the difference in the substomatal carbon dioxide concentration was significant between control group and drought treated groups (Fig. 4a).

The maximum photosynthesis at elevated CO_2 (1500 ppm) and high irradiance (1.5 mmol m^{-2} s^{-1}) was higher in stressed seedlings (there was a statistically significant ($p < 0.05$) difference between control group and 15% group; Fig. 3b). The same was true for the ratio of photosynthesis and substomatal carbon dioxide concentration under high irradiance and normal CO_2 concentrations (there was a statistically significant ($p < 0.05$) difference between control group and 15% group; Fig. 5), which according to Long and Bernacchi (2003) indicates on the restriction of photosynthesis caused by Rubisco activity.

The F_V/F_M ratio measuring the maximum yield of PSII photochemistry (Fig. 6a), was higher in stressed seedlings than in the control group. Both stressed groups differed significantly from the control group in F_V/F_M ratio ($p < 0.05$), but

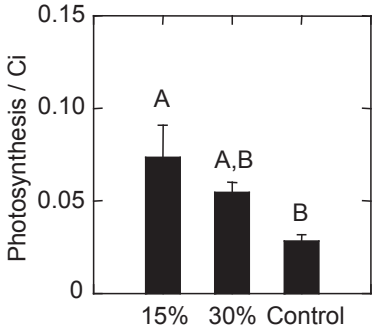

Fig. 5. Ratio of photosynthesis and intercellular CO_2 concentration (Ci) under normal CO_2 conditions (360 ppm). Lines in the bars represent the standard errors of mean. Means marked with different letters differ statistically from each other at $p < 0.05$, Tukey's test.

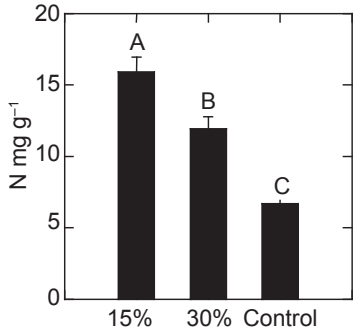

Fig. 7. Differences in mass-based nitrogen (mg g^{-1}) between treatments. Lines in the bars represent the standard errors of mean. All means differ statistically from each other at $p < 0.05$, Tukey's test.

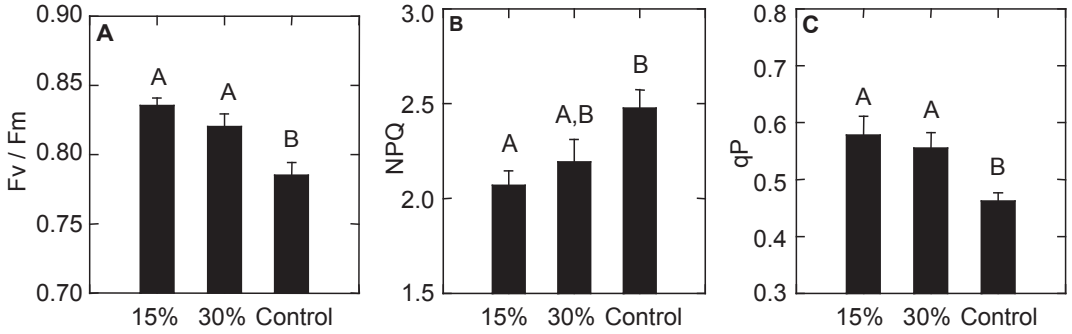

Fig. 6. (A) The F_V /F_M ratio, (B) non-photochemical quenching (NPQ) and (C) photochemical quenching (qP). Lines in the bars represent the standard errors of mean. Means marked with different letters differ statistically from each other at $p < 0.05$, Tukey's test.

there was no statistically significant difference between the drought stressed groups ($p > 0.05$). The proportion of open PSII centres (qP) was far larger in stressed groups (results were statistically significant between the control treatment and the stressed treatments but the stressed treatments did not differ statistically from each other), which indicates that electron transport from PSII increased with stress (Fig. 6c). At the same time non-photochemical quenching (NPQ, Fig. 6b) decreased (there was a statistically significant difference between control and 15% drought treatment).

Nitrogen concentration in the leaves was relatively low (between 0.7–1.5% on a leaf mass basis) and it increased with the drought stress (the difference between groups was statistically significant at $p < 0.05$, Fig. 7). There was no significant correlation between SLA and photosynthesis rate in high CO_2 concentration but SLA and leaf nitrogen concentration correlated with each other (Pearson correlation coefficient 0.761, $p < 0.05$, data not presented). The nitrogen per leaf area was higher in the drought stressed groups, but differences were reduced when the amount of leaf nitrogen was divided by the fine root biomass (Table 1).

4 Discussion

The drought stress affected the biomass production and growth of seedlings profoundly: drought stressed seedlings were significantly smaller than unstressed seedlings (Table 1). Root/shoot ratio increased as a result of the drought (Table 1). Differences in the productivity of leaves, as measured by net photosynthesis, were less pronounced (Table 1).

4.1 Growth and Morphology

As expected (Boyer 1982), the total biomass of seedlings was lower in the drought treatments. In other words, growth rates decreased with drought (Table 1). At the same time there was a shift in root mass towards finer roots that take up the bulk of the water (Table 1). Also the root/shoot ratio was higher in seedlings belonging to the stressed treatments than in control group.

One might argue that these differences were an artefact due to an insufficient pot size used in the experiment, which might have inhibited the root growth of the larger seedlings. In our experiment roots did not fill the pot at the end of the experiment so we do not believe that pot size (2 l) limited root growth. According to earlier results, the root/shoot ratio of *Eucalyptus* increases usually under drought, but there are exceptions (Bachelard 1986; Osório et al. 1998). Fine root mass to leaf area ratio gives an idea on the ratio between nutrient uptaking organs and carbon uptaking organs and may be a better indicator of the equilibrium between carbon and nutrient requirements. Drought stressed seedlings had a higher fine root / leaf area ratio than non-stressed seedlings, although the differences were not statistically significant (Table 1).

Specific leaf area (SLA) was significantly larger in stressed than in control seedlings and it increased with the degree of stress (Table 1). In the studies of Li (1999) and Tuomela (1997) the seedlings of *E. microtheca* behaved the opposite way or there were no drought induced changes in SLA (Li 1999). SLA and nitrogen concentration of leaves correlate usually, as they did in our data. Earlier studies have proven that SLA and nitrogen in *Eucalyptus* species can correlate either positively or negatively with each other (Prior et al. 2003; Eamus et al. 1999; Sefton et al. 2002; Grassi et al. 2002). Schulze et al. (1998) claimed that even though in most cases leaf N and SLA correlate positively, there are differences in the reactions between species.

Nitrogen concentrations in our data were relatively low, ranging between 0.7 and 1.5% (Fig. 7). However, they are in line with a number of published studies that found similar concentrations for Australian savannah trees. Schulze et al. (1998) reported nitrogen concentrations between 0.7 and 1.2% in evergreen non-N_2 fixing trees and Prior et al. (2003) found nitrogen concentrations between 0.7 to 2.3% depending on *Eucalyptus* species. They found that nitrogen concentrations are significantly lower in evergreen than deciduous species and also lower in woodland species than in open forests or dry monsoon forest species. Farquhar et al. (2002) have noted that nitrogen concentration in *E. dichromophloia* leaves increases with decreasing mean annual rainfall.

We acknowledge that there exists a risk that our fertilization regime was responsible for changes in the N status between the treatments, as the seedlings were fertilized according to the mean size of all seedlings, whereas the mean size of the seedlings in each treatment group varied. However, root/shoot ratios, which usually increase under nitrogen deficits, were smaller in the control treatment. Since root/shoot ratio are normally responsive to N deficiencies we think that higher fine-root / leaf area ratios show that nitrogen deficiency was not a very important factor in the control treatment. Furthermore, our N values were similar to the values in the field. We investigated on what extent the changes in the root/shoot ratio could explain the differences in N contents. When calculating the amount of nitrogen in leaves per fine root mass (as a measure of how much nitrogen the plant has taken up per unit of root biomass) the differences between the treatments are reduced greatly. All this indicates that differences in the nitrogen content of leaves are probably primarily caused by different allocation strategies, which were modulated by drought.

4.2 Physiology

The roots of *Eucalyptus* trees are usually well developed in the dry areas and the depths of the soil roots are penetrating in enables them to use the water stored deep in the soil during the dry season. Therefore, the transpiration rates per unit leaf area in the field during the dry season are similar to those in the wet season in some *Eucalyptus* species, since evaporative demand is higher in the dry season (O´Grady et al. 1999). In greenhouse experiments with seedlings, however, transpiration usually decreases since the evaporative demand does not change over the growing season (i.e. Pereira and Kozlowski 1976; Li et al. 2000). Based on an experiment made with *E. camaldulensis* seedlings from dry tropics, Gibson et al. (1995) proposed that the seedlings depend much more upon the reduction in leaf area than on the stomatal control of transpiration to conserve water. Generally, reductions of transpiration in greenhouse experiments were larger than in field experiments, probably because field grown adult trees have deep rooting systems and, often, access to ground water.

We think that drought stress did not change the hydraulic permeability of xylem, since stressed and unstressed plants did not differ from each other on this matter (Fig. 2). Therefore it is probable that embolism was not important in the drought stressed seedlings, since embolism reduces usually hydraulic permeability. In a study made with *E. camaldulensis*, the permeabilities of stem wood in the seedlings from semi-arid areas were consistently higher than in seedlings from humid areas. To induce a significant degree of embolism very negative values of water potential were required (Franks et al. 1995) and it is possible that the drought treatments in our experiment were not drastic enough to induce embolism.

The rate of photosynthesis is usually significantly lower in plants suffering from water deficit than in those with an adequate supply of water. If species do not change their stomatal conductance during dry periods, they would have to build a larger root system to access soil water and to be able to continue photosynthesis and transpiration (e.g. Sinclair 1980; White et al. 2000). In this study no statistically significant differences between treatments in the net photosynthesis or stomatal conductance were found (Fig. 3A and Fig. 4B). Stomatal conductance was, nevertheless, two times higher in the control treatment than in the 15% treatment. The differences in the transpiration rates between the treatments (Table 1, Fig. 1) indicate increases in stomatal limitation with drought, even though the response in the stomatal limitation varied much between the seedlings (Fig. 4B). Altogether, the results deviate from "normal response of plants to drought" which seems to be a slow downscaling of the biochemical capacity of photosynthesis as a response to drought (i.e. the Meta-Analysis of Flexas and Medrano 2002b). However, in an earlier review Kaiser (1987) showed that decreases in photosynthesis do not necessary occur as a response to moderate water stress. The results of this study differed from those reported by Li (2000) about several provenances of *E. microtheca*. In his study, water stress (six months old seedlings kept at 25% of field capacity) clearly lowered the amount of net photosynthesis. *Eucalyptus* species are known to have wide variation in the stomatal sensitivity to drought (e.g. Sinclair 1980; White et al. 2000), and according to our results it would seem that stomatal sensitivity can vary much even within species and possibly also between leaves of a plant.

The fluorescence F_V/F_M ratio is a good indicator of photoinhibition, because it correlates with the quantum yield of O_2 evolution (Björkman and Demmig 1987). It is usually thought that F_V/F_M ratio should decrease during environmental stresses (Krause and Weis 1991). Values of F_V/F_M below 0.8 are usually interpreted as an indication of photoinhibitory stress (Maxwell and Johnson 2000). In our study the F_V/F_M increased with the drought stress (Fig 6A). Previous studies found no decrease in F_V/F_M ratio with water stressed durum wheat (*Triticum turgidum* var. *durum*) (Tambussi et al. 2002) and Sánchez-Rodriguez et al. (1997) even found an increase of F_V/F_M ratio with *Casuarina equisetifolia*, although prolonged droughts decreased the F_V/F_M ratios. The average values within treatments in this study were between values suggested by Bolhàr-Nordenkampf and Öquist (1993). According to the results of this study the ability of *E. microtheca* to absorb quanta is increased during drought stress, as both heat dissipation (NPQ, Fig. 6B) and photoinhibition

(Fig. 6A) were lower in drought stressed groups. As the growth of the seedlings was highest in the control group, these results are not a product of a too high irrigation rate. Also we did not observe large amount of dead fine roots in the control group indicating that our seedlings did not suffer from anoxia.

Our results indicate furthermore that electron transport from PSII (qP) through photochemical processes increased with drought (Fig. 6C) and at the same time non-photochemical quenching (NPQ), describing the loss of quantum yield as heat, decreased (Fig. 6B). Sánchez-Rodriguez et al. (1997) found also, that after 33 days of drought treatment F_V/F_M ratio in *Casuarina equisetifolia* increased slightly. Though, with *C. equisetifolia* the photoprotection occurred as increased heat dissipation, not as increased energy transfer through photochemical processes that occurred in our study.

Under saturated concentrations of substomatal CO_2 and saturated light the rate of assimilation describes the maximum capacity of leaves to regenerate RuBP (e.g. Long and Bernacchi 2003). RuBP regeneration decreases usually during drought and is thought to be the main factor limiting carbon assimilation (Gunasekera and Berkowitz 1993; Flexas and Medrano 2002a). Our results do not support these assumptions. The net photosynthesis under high CO_2 – describing the regeneration rate of RuBP clearly increased in the stressed seedlings (Fig. 3B) and still the net photosynthesis remained at the same level in the stressed seedlings as it was in well-watered seedlings (Fig. 3A).

Warren et al. (2000) found in their study of several Australian tree species, that in most cases the actual concentration of Rubisco was far larger than necessary for supporting the measured rates of photosynthesis. This suggests that many native Australian tree species may have Rubisco in excess to their needs under unstressed conditions (Warren et al. 2000). Warren et al. (2000) argued further that the excess Rubisco might be an advantage under drought and low phosphorous conditions, as they often occur in tropical Australia. Gibson et al. (1991) found out that the nitrogen content increased during drought treatment in some *E. camaldulensis* provenances. The nitrogen content of leaves is known to correlate well with

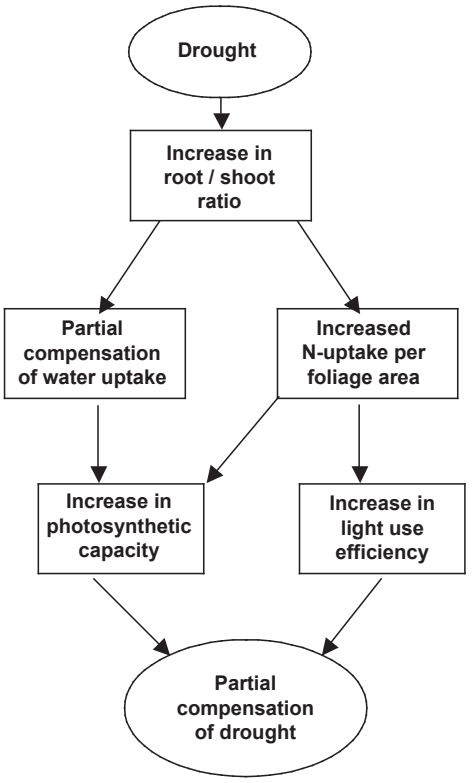

Fig. 8. Compensation of drought in *E. microtheca*.

Rubisco (Woodrow and Berry 1988) and increases in Rubisco (and nitrogen) will make plants more resistant against photoinhibitory damage. Also, based on theoretical arguments Farquhar et al. (2002) propose that nitrogen concentrations in leaves should increase under drought.

To summarize the photosynthetic reactions, *E. microtheca* does not scale down its photosynthesis as a reaction to drought, but it seems to increase its photosynthetic capacity while reducing intercellular CO_2 concentration (Ci). Protection against photoinhibition seems to be maintained by a higher Rubisco activity and by diverting absorbed irradiance through photochemical quenching.

We conclude that *E. microtheca* is using allocation between foliage and roots as the key mechanism to survive under drought. As a response to drought the seedlings increase their root/shoot ratios. This compensates for some of the negative effects of drought and should enable the plant to

maintain much of its water uptake under moderate drought. Furthermore the increase in root biomass will increase foliage nitrogen concentrations and would increase photosynthesis under a given substomatal CO_2 concentration. This strategy is graphically represented in Fig. 8.

The strategy would agree with the theoretical consideration of Farquhar et al. (2002) and Buckley et al. (2002) that propose that increases in leaf N and increased allocation of N to Rubisco would represent an optimal strategy for plants under drought. This strategy would make it possible to react rapidly to changes in soil water availability (i.e. after thunderstorms) since no time consuming reconstruction of the photosynthetic system is required. The fact that Australian plants have high amounts of Rubisco supports these conclusions (Warren et al. 2000). Also, this could explain, how Australian savannah plants can maintain high rates of transpiration during the dry season (O´Grady et al. 1999). This kind of opportunistic water use strategy could be a reasonable and frequent strategy where droughts are common but not very predictable.

Altogether, the current study shows that different mechanisms for drought tolerance are highly interconnected. Changes in allocation to the root system make it possible for plants to maintain a high photosynthetic capacity due to photoprotection and high activities of Rubisco but at the cost of a reduced leaf growth. Field tests will have to show to what extent this strategy is relevant in nature for Australian evergreen tree species.

Acknowledgements

We would like to thank Dr. Chunyang Li for help, Dr. Pertti Hari for borrowing the photosynthesis machine and Daniel Richterich for the help with the work in the greenhouse.

References

Bachelard, E.P. 1986. Effects of soil moisture on the growth of seedlings of three eucalypt species. II growth effects. Australian Forest Research 16: 51–61.

Björkman, O. & Demmig, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants from diverse origins. Planta 170: 489–504.

Bolhàr-Nordenkampf, H.R. & Öquist, G. 1993. Chlorophyll fluorescence as a tool in photosynthesis research. In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C. & Long, S.P. (eds). Photosynthesis and production in a changing environment: a field and laboratory manual. Chapman & Hall, London. p. 193–205.

Bota, J., Medrano, H. & Flexas, J. 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress. New Phytologist 162: 671–681.

Boyer, J.S. 1982. Plant productivity and environment. Science 218: 443–448.

Buckley, T.N., Miller, J.M. & Farquhar, G.D. 2002. The mathematics of linked optimalisation for water and nitrogen use in a canopy. Silva Fennica 36: 639–669.

Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. Journal of Experimental Botany 42: 1–16.

Collatz, J., Ferrar, P.J. & Slatyer, R.O. 1976. Effects of water stress and differential hardening treatments on photosynthetic characteristics of a xeromorphic shrub, Eucalyptus socialis, F. Muell. Oecologia 23: 95–105.

CSIRO. 1979. Seeds section report no 1. Division of Forest research, CSIRO, Canberra.

Damesin, C. & Rambal, S. 1995. Field study of leaf photosynthetic performance by a Mediterranean deciduous oak tree (Quercus pubescens) during a severe summer drought. New Phytologist 131: 159–167.

Eamus, D., Myers, B., Duff, G. & Williams, D. 1999. Seasonal changes in photosynthesis of eight savanna tree species. Tree Physiology 19: 665–671.

FAO 1979. Eucalypts for planting. FAO Forestry Series. Food and agriculture organization of the United Nations, Rome.

Farquhar, G.D., Buckley, T.N. & Miller, J.M. 2002.

Optimal stomatal regulation model and a biochemical model in explaining CO_2 exchange in field conditions. Silva Fennica 36: 625–637.

Flexas, J. & Medrano, H. 2002a. Drought inhibition of photosynthesis in C_3 plants: Stomatal and non-stomatal limitations revisited. Annals of Botany 89: 183–189.

— & Medrano, H. 2002b. Energy dissipation in C_3 plants under drought. Functional Plant Biology 29: 1209–1215.

Franks, P.J., Gibson, A. & Bachelard, E.P. 1995. Xylem permeability and embolism susceptibility in seedlings of Eucalyptus camaldulensis Dehnh. from two different climatic zones. Australian Journal of Plant Physiology 22: 15–21.

Gibson, A., Hubick, K.T. & Bachelard, E.P. 1991. Effects of abscisic acid on morphological and physiological responses to water stress in Eucalyptus camaldulensis Seedlings. Australian Journal of Plant Physiology 18: 153–163.

— , Bachelard, E.P. & Hubick, K.T. 1995. Relationship between climate and provenance variation in Eucalyptus camaldulensis Dehnh. Australian Journal of Plant Physiology 22: 453–460.

Grassi, G., Meir, P., Cromer, D., Tompkins, D. & Jarvis, P.G. 2002. Photosynthetic parameters in seedlings of Eucalyptus grandis as affected by rate of nitrogen supply. Plant, Cell & Environment 25: 1677–1688.

Gunasekera, D. & Berkowitz, G.A. 1993. Use of transgenic plants with ribulose-1,5-bisphosphate carboxylase/oxygenase antisense DNA to evaluate the rate limitation of photosynthesis under water stress. Plant Physiology 103: 629–635.

Kaiser, W. 1987. Effects of water deficit on photosynthetic capacity. Physiologia Plantarum 71: 142–149.

Krause, G.H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annual Review of Plant Physiology and Plant Molecular Biology 42: 313–349.

Li, C. 1999. Carbon isotope composition, water use efficiency and biomass productivity of Eucalyptus microtheca populations under different water supplies. Plant and Soil 214: 165–171.

— 2000. Population differences in water-use efficiency of Eucalyptus microtheca seedlings under different watering regimes. Physiologia Plantarum 108: 134–139.

— & Wang, K. 2003. Differences in drought responses of three contrasting Eucalyptus microtheca F. Muell populations. Forest Ecology and Management 179: 377–385.

— , Berninger, F., Koskela, J. & Sonninen, E. 2000. Drought responses of Eucalyptus microtheca provenances depend on seasonality of rainfall in their place of origin. Australian Journal of Plant Physiology 27: 231–238.

Long, S.P. & Bernacchi, C.J. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. Journal of Experimental Botany 54: 2393–2401.

Maxwell, K. & Johnson, G.N. 2000. Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany 51: 659–668.

O´Grady, A.P., Eamus, D. & Hutley, L.B. 1999. Transpiration increases during the dry season: Patterns of tree water use in eucalypt open-forests of northern Australia. Tree Physiology 19: 591–597.

Osório, J., Osório, M.L., Chaves, M.M. & Pereira, J.S. 1998. Water deficits are more important in delaying growth than in changing patterns of carbon allocation in Eucalyptus globulus. Tree Physiology 18: 363–374.

Pereira, J.S. & Kozlowski, T.T. 1976. Leaf anatomy and water relations of Eucalyptus camaldulensis and E. globulus seedlings. Canadian Journal of Botany 54: 2868–2880.

Prior, L.D., Eamus, D. & Bowman, D.M.J.S. 2003. Leaf attributes in the seasonally dry tropics: a comparison of four habitats in northern Australia. Functional Ecology 17: 504–515.

Sánchez-Rodríguez, J., Martínez-Carrasco, R. & Pérez, P. 1997. Photosynthetic electron transport and carbon-reduction-cycle enzyme activities under long-term drought stress in Casuarina equisetifolia Forst. & Forst. Photosynthesis Research 52: 255–262.

Schulze, E.-D., Williams, R.J., Farquhar, G.D., Schulze, W., Langridge, J., Miller, J.M. & Walker, B.H. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Australian Journal of Plant Physiology 25: 413–425.

Sefton, C.A., Montagu, K.D., Atwell, B.J. & Conroy, J.P. 2002. Anatomical variation in juvenile eucalypt leaves accounts for differences in specific leaf area and CO_2 assimilation rates. Australian Journal of Botany 50: 301–310.

Sinclair, R. 1980. Water potential and stomatal conductance of three Eucalyptus species in the Mount Lofty Ranges, South Australia: Responses to summer drought. Australian Journal of Botany 28: 499–510.

Tambussi, E.A., Casadesus, J., Munné-Bosch, S. & Araus, J.L. 2002. Photoprotection in water-stressed plants of durum wheat (Triticum turgidum var. durum): changes in chlorophyll fluorescence, spectral signature and photosynthetic pigments. Functional Plant Biology 29: 35–44.

Tezara, W., Mitchell, V.J., Driscoll, S.D. & Lawlor, D.W. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401: 914–917.

Tuomela, K. 1997. Physiological and morphological responses of Eucalyptus microtheca provenances to water availability in tropical drylands. Tropical Forestry Reports 13.

Warren, C.R., Adams, M.A. & Chen, Z. 2000. Is photosynthesis related to the concentrations of nitrogen and Rubisco in leaves of Australian native plants. Australian Journal of Plant Physiology 27: 407–416.

White, D.A., Turner, N.C. & Galbraith, J.H. 2000. Leaf water relations and stomatal behaviour of four allelopathic Eucalyptus species planted in Mediterranean southwestern Australia. Tree Physiology 20: 1157–1166.

Whitehead, D., Jarvis, P.G. & Waring, R.H. 1984. Stomatal conductance, transpiration, and resistance to water uptake in a Pinus sylvestris spacing experiment. Canadian Journal of Forest Research 14: 692–700.

Woodrow, I.E. & Berry, J.A. 1988. Enzymatic regulation of photosynthetic CO_2 fixation in C3 plants. Annual Review of Plant Physiology and Plant Molecular Biology 39: 533–594.

Total of 43 references