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Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings

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Abstract

Water and nutrients are two of the most important factors controlling the growth of trees. Numerous studies show that fertilization is most effective when trees are not water-stressed and that irrigation is most effective when nutrients are not scarce. The goal of this work was to test the responses of *Eucalyptus grandis* seedlings to drought as affected by soil type and fertilization with nitrogen (N) or phosphorus (P). Seedlings of *E. grandis* were planted in pots with two different types of soil: a sandy soil and a clay (black) soil. Plants were fertilized with N or P, and half of them were water-stressed. Fertilization did not affect the growth reduction brought about by drought in the red sands; in the black soil, plants fertilized with P showed a moderate growth increase under drought, compared to non-fertilized plants. Dry mass allocation to roots was decreased by fertilization in water-stressed and well-watered plants in the sandy soil, and in well-watered plants of the black soil, but it increased in water-stressed plants fertilized with N in the black soil. Water use efficiency (WUE), i.e., the grams of water transpired per gram of dry mass accumulated, was not altered by fertilization or water stress in the black soil, although it increased with P fertilization in well-watered plants in the sandy soil. Osmotic adjustment was observed only in plants fertilized with P in the black soil, and in non-fertilized plants in the sandy soil. Osmotic adjustment in water-stressed plants was associated with an increased stomatal conductance. We conclude that water stress tolerance strategies are altered by fertilization depending on soil properties, and that fertilization with P is recommended in black soils even if a moderate drought is likely to occur, but on sandy soils fertilization is recommended only under good water supply.

Keywords: Drought; Osmotic adjustment; Pressure–volume curve; Soil/fertilization interactions; Stomatal conductance; Water use efficiency

1. Introduction

Water and nutrients are two of the most important factors controlling the growth of trees (Stoneman et al., 1996). Numerous studies show that fertilization is most effective when trees are not water-stressed, and that irrigation is most effective when nutrients are not scarce (Sands and Mulligan, 1990). *Eucalyptus* productivity is strongly influenced by nutrient and water availability (Misra et al., 1998), but at sites in Mediterranean climates, irrigation increases produc-

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tivity more than nutrient supply without irrigation (Madeira et al., 2002). Moreover, at sites with adequate rainfall but without a well-textured soil, the availability of water, more than nutrients, is likely to determine the growth of trees (Judd et al., 1996). Growth reduction by drought is an important cause of yield reduction, but survival after planting is equally important for plantations productivity (Pita and Prados, 2001). The current expansion of plantations to sites with rainfall near the limits of the species ability to survive and grow makes resistance to water stress an important attribute of seedlings (Sasse and Sands, 1996; Whitehead and Beadle, 2004), and therefore, it is important to know how common silvicultural practices, like fertilization, affect seedling resistance to drought.

Fertilization at planting is a common practice in eucalypt plantation programs. In Australia, 10–25 g of nitrogen (N) and around 25 g of phosphorus (P) per tree are added at planting, or shortly thereafter (Cromer, 1996). The standard fertilization practice in New Zealand involves 27.6 g of N per tree on uncultivated sites and 13.8 g per tree on machine-cultivated sites, 3 months after planting and a similar application a year later (Knight and Nicholas, 1996). Optimum applications for different locations in South Africa vary between 30 and 62 kg of N ha^{-1} and between 10 and 37 kg of P ha^{-1} depending on soil type, soil condition and organic matter content (Herbert, 1996). In Brazil, applications of 25–75 kg of N ha^{-1} and 64–70 kg of P ha^{-1} are recommended depending on site productivity (Barros and Novais, 1996; Barros et al., 1990). The common fertilization practice in Argentina is to apply around 50 kg of N ha^{-1} and 58 kg of P ha^{-1} at planting (Dalla Tea and Marcó, 1996).

Several environmental factors interact with low water availability to determine the extent of drought-induced growth reductions (White et al., 1996). Plants have developed various mechanisms to withstand drought, but the biological cost of these mechanisms may differ in terms of productivity (Pita and Prados, 2001). There is not much information on the effect of fertilization on susceptibility to drought in trees (Fisher and Binkley, 2000). Eucalypt seedlings and other tree species subjected to moderate, long-term water stress can respond by developing higher root–shoot ratios, fewer and smaller leaves, or by increasing the concentration of solutes in leaf cells (Snowdon, 2000). Fertilization can affect drought tolerance through alterations in dry matter partitioning, because trees invest relatively less assimilates into roots and more into leaves when nutrients are not limiting (Sands and Mulligan, 1990). Thus, changes in water and nutrient use efficiency can be observed in fertilized plants. Fertilization can also affect xylem hydraulic conductivity. This is observed in poplar with N fertilization increasing the vulnerability of xylem to cavitation, whereas P fertilization reduced cavitation vulnerability (Harvey and Driessche, 1997). The reduction in hydraulic conductivity caused by nutrient deficit can be reversed by fertilization (Sands and Mulligan, 1990), and this might affect drought responses. Finally, the accumulation of solutes to decrease water potential, i.e., osmotic adjustment (Dichio et al., 2003), may allow plants to maintain a water potential gradient as the soil becomes drier. This allows plants to maintain the positive leaf turgor required to keep stomata open and sustain gas exchange and growth (White et al., 2000). The capacity for osmotic adjustment may depend on nutrient availability, with fertilized chestnut oak seedlings exhibiting greater osmotic adjustment than plants with lower nutrient availability (Kleiner et al., 1992).

Fertilization at the time of planting is a common practice in *Eucalyptus* plantations, but it is not well known how fertilization alters seedling tolerance to drought. The objective of this work was to assess the effects of fertilization with N or P on the responses of *Eucalyptus grandis* seedlings to drought imposed on two very different types of soil. Changes in dry matter partitioning, water use efficiency (WUE), stomatal conductance and osmotic adjustment in response to water stress, fertilization and soil type were determined.

2. Materials and methods

2.1. Plant material and soil type

Three-month-old *E. grandis* seedlings were transplanted into 4-l pots (approximately, 6 kg of soil) that contained either of two types of soils characteristic of Entre Ríos province, one of the most important eucalypt production areas in Argentina (Dalla Tea and

Marcó, 1996). These soils are a deep red sandy soil (Oxic Quartzipsamment, referred to as "red sands" in this paper) and a black soil (Argiudolic Pelludert, hereafter referred to as "black soil"). On these soils *E. grandis* produces 328 and 256 m^3 ha^{-1}, respectively, in 10-year-old plantations (Dalla Tea and Marcó, 1996; Goya et al., 1997). Soil chemical analyses were performed using the following methods: pH in water 1:2.5 (w/v), electric conductivity in soil paste, P by the Kurtz and Bray method, carbon by the Walkely Black method, N by microKjeldhal, and calcium, magnesium, sodium, potassium and cation exchange capacity (CEC) in ammonium acetate (pH 7) by atomic absorption (Departamento de Suelos, FAUBA, Universidad de Buenos Aires, Argentina). For each type of soil, curves relating soil water content with soil water potential were fitted in order to know the gravimetric water content at which water potential equalled −0.03 and −0.8 MPa. Curves were fitted measuring the gravimetric water content that balanced pressures of 0, 0.05, 0.1, 0.3, 0.5 and 0.8 MPa according to the pressure plate method (Richards et al., 1956).

2.2. Fertilization and water treatments

Ten replicate plants were randomly allocated to each treatment in an experiment that combined the two soil treatments with three fertilizer (with N or P and without fertilizer) and two water availability treatments (well-watered and water-stressed). Eucalypts planted in each type of soil were fertilized either with N or P. Doses applied were the optimal for each type of soil according to a previous fertilization experiment (Graciano and Goya, unpublished observations). N was applied at the rate of 1 and 2 g of urea (46-0-0) per plant (corresponding to 0.46 and 0.92 g of N) in the red sands and black soil, respectively. P was applied as 6 g of calcium super phosphate (48-0-0) in the red sands (2.88 g of PO_5) and as 12 g of the same fertilizer in the black soil (5.76 g of PO_5). Ten control pots without fertilization were included for each type of soil. Fertilizers were applied at transplanting; the water stress treatment was started 5 days thereafter. In order to reach a similar level of stress in both types of soil, the soil of 10 pots of each fertilizer treatment was maintained at −0.8 MPa throughout the experiment, while another set of 10 plants was kept at field capacity (−0.03 MPa) to serve as controls. To prevent evaporation from the soil, pots were placed in plastic bags filled with expanded white polystyrene beads that covered the soil surface completely. Pots were weighed every other day during the 20 days of the water stress treatment to estimate daily transpiration. After weighing, pots were watered to their target soil water contents. Well-watered plants were watered to recover field capacity, i.e., 6.4% of gravimetric water content in the red sands and 39.4% in the black soil. The stressed plants were watered only to reach −0.8 MPa, i.e., 1.5% of gravimetric water content in the red sands and 28.4% in the black soil (Fig. 1). The interference by the change in plant weight during the experiment was negligible and was not taken into account in these calculations.

Plants grew outdoors during November–December (late spring–early summer for the Southern Hemisphere). Plant dry weight was measured at the beginning of the experiment on a randomly chosen sample of 10 plants. At the end of the experiment, all the plants of each treatment ($n = 10$) were also weighed dry. Plant material was dried in an oven at 60 °C to reach constant weight. The increase in dry weight was calculated by subtracting final total dry weight minus the mean initial dry weight. Water use

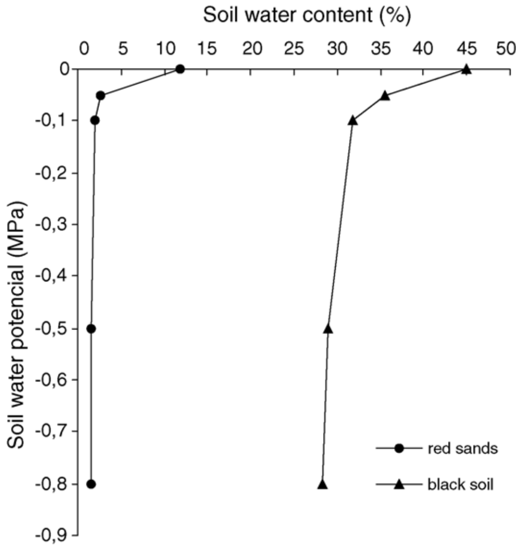

Fig. 1. Relationship between percentage soil water content (%) and soil water potential (MPa) in the red sands and the black soil.

efficiency was calculated by dividing the increase in total dry mass by the weight of water transpired during the experiment. Plants were divided into leaves, branches, stems and roots in order to evaluate dry matter partitioning.

2.3. Stomatal conductance and pressure–volume curves

At the end of the experiment, 20 days after the start of the water availability treatment, six plants per treatment were randomly chosen for measurements of stomatal conductance. The next morning, six plants per treatment were randomly chosen to determine pressure–volume curves. At the end of these measurements, all 10 plants of each treatment were dried for dry mass measurements. Stomatal conductance of the uppermost fully expanded leaf was measured at midday under sunny skies with a steady state porometer (LI-1600, Licor, USA). Pressure–volume curves were fitted by measuring relative water content (RWC) at different values of water potential on six terminal branches per treatment (Tyree and Richter, 1982). Water potential of the branches was measured with a pressure chamber (Cochard et al., 2001), and branches were weighed immediately after each balance pressure reading in order to calculate the relative water content. Measurements were started early in the morning immediately after cutting off the branches from the plant. Branches were then kept for 2 h in plastic bags with their cut ends in water to reach full turgor, and another measurement was made. Thereafter, two additional measurements were made after allowing branches to transpire freely in the lab for 1–2 h. After completing the readings, plant material was oven-dried to constant mass in order to calculate relative water content. For each reading, RWC was calculated as:

$$\mathrm{RWC} = \left(\frac{\text{fresh weight} - \text{dry weight}}{\text{full turgor fresh weight} - \text{dry weight}}\right) \times 100$$

Curves were fitted using the Pressure–Volume Analysis Program (Paul J. Schulte, 1998, University of Nevada, Las Vegas), with a parameter error tolerance of 0.01. Osmotic potential at turgor loss point, osmotic potential at saturation and relative water content at turgor loss point were derived from the curves.

2.4. Statistical analysis

Data were analyzed by MANOVA with soil type, fertilizer treatments and water availability treatments as main factors. Means differences were compared with Tukey's test ($P < 0.05$).

3. Results

3.1. Soil analysis

The two soil types used in this work have very different physical and chemical characteristics, and different water retention behavior. The black soil has higher carbon content, P and N concentrations, as well as higher calcium, magnesium and potassium availability with a higher exchangeable cation capacity (Table 1). The texture of both soils is very different, as can be seen in their proportions of clay, silt and sand. Therefore, the black soil has higher nutrient availability and a greater capacity to store water than the red sands (Fig. 1).

Table 1
Physical and chemical characteristics of the two soils used in this work

Local name	Red sands	Black soil
US classification	Oxic Quartzipsamment	Argiudolic Pelludert
pH	6.44	6.92
Electrical conductivity (dS m^{-1})	0.08	0.9
P (ppm)	3.74	7.99
C (%)	0.45	1.67
Total N (%)	0.03	0.15
Ca (meq $(100\ g)^{-1}$)	1.78	18.12
Mg (meq $(100\ g)^{-1}$)	0.46	1.65
Na (meq $(100\ g)^{-1}$)	0.23	0.33
K (meq $(100\ g)^{-1}$)	0.31	1.14
CEC (meq $(100\ g)^{-1}$)	6.95	23.14
% Clay	7.5	42.5
% Silt	5	27.5
% Sand	87.5	30
Soil texture	Sand	Silt clay loam
Apparent density (g cm^{-3})	1.60	0.97

Methods used for determinations were: pH in water 1:2.5, electric conductivity in soil paste, P by the Kurtz and Bray method, carbon by the Walkely Black method, N by microKjeldhal, and calcium, magnesium, sodium, potassium and cation exchange capacity (CEC) in ammonium acetate (pH 7).

Table 2
Total dry mass increase (g) and root:total plant mass ratio for the non-fertilized (−F) and fertilized (+N and +P) treatments in the two types of soil, with and without water stress

	Total dry mass increase (g)		Root:total	
	Well-watered	Water-stressed	Well-watered	Water-stressed
Red sands				
−F	9.30 ± 1.00 bc	4.18 ± 0.40 d	0.37 ± 0.01 b	0.45 ± 0.02 a
+N	12.06 ± 1.49 ab	4.45 ± 0.60 d	0.31 ± 0.01 cd	0.34 ± 0.01 c
+P	15.66 ± 0.54 a	5.94 ± 0.30 cd	0.27 ± 0.01 e	0.28 ± 0.01 de
Black soil				
−F	12.76 ± 0.80 b	4.67 ± 0.30 d	0.32 ± 0.01 a	0.25 ± 0.01 b
+N	17.55 ± 0.90 a	6.42 ± 0.97 cd	0.24 ± 0.01 b	0.33 ± 0.01 a
+P	19.76 ± 1.32 a	8.43 ± 0.63 c	0.20 ± 0.01 c	0.24 ± 0.01 b

Values are the mean ± standard errors. For each type of soil, means followed by the same letters (a–e) are not significantly different at $P < 0.05$.

3.2. Plant growth and water use efficiency

Total dry mass accumulation was 56–64% lower in water-stressed than in well-watered plants in both soils, indicating that plants experienced a moderate water stress. Fertilization caused an increase of dry mass accumulation in well-watered plants in both soils (although not significantly for N in the red sands) (Table 2). Addition of N or P produced no dry mass increase in the stressed plants in the sandy soil, whereas P fertilization increased dry matter accumulation in stressed plants in the black soil (Table 2). Dry mass partitioning did not change much either with the stress or fertilization treatments. Therefore, a close linear relationship was found between the dry mass of leaves, branches and stems, and total plant dry mass across soil types, fertilization and water availability treatments (Fig. 2). This linear relationship was not as strong for dry mass of roots and total plant dry mass. Fertilization with N or P decreased the root:total plant dry mass ratio in well-watered plants in both soils. In the red sands, fertilization also reduced the root:total plant mass ratio under water stress (Table 2). In the black soil, under water stress, N application increased allocation to roots and P application did not change root:total ratio. Therefore, in general fertilization tended to decrease the root:total plant dry mass ratio.

Daily transpiration was about three-fold higher in well-watered than in water-stressed plants in both soils (Table 3). Fertilization increased transpiration in well-watered plants in the black soil. However, fertilization did not alter water use efficiency in either stressed or watered plants because increased transpiration correlated with higher dry mass accumulation in fertilized, well-watered plants (Tables 2 and 3). N fertilization increased transpiration under water deficit in the black soil. In the red sands, P and N applications increased daily transpiration in well-watered plants, and P also increased WUE. Fertilization had no effect on transpiration or WUE of water-stressed plants in the red sands.

3.3. Stomatal conductance and osmotic adjustment

Stomatal conductance was 2.5–8-fold lower in stressed than in well-watered plants (Table 4). Fertilization treatments did not change stomatal

Table 3
Mean daily transpiration (g H_2O day^{-1}) and water use efficiency (WUE) (g dry matter (DM) g^{-1} water transpired) for the non-fertilized (−F) and fertilized (+N and +P) treatments across the two types of soil, with and without water stress

	Mean daily transpiration (g d^{-1})		WUE (mg DM g^{-1} water)	
	Well-watered	Stressed	Well-watered	Stressed
Red sands				
−F	82 ± 2 b	30 ± 2 c	5 ± 0.6 b	6 ± 0.4 ab
+N	100 ± 7 a	37 ± 3 c	6 ± 0.5 ab	7 ± 0.6 ab
+P	101 ± 5 a	35 ± 2 c	7 ± 0.5 a	8 ± 0.6 a
Black soil				
−F	69 ± 4 b	24 ± 2 d	10 ± 0.8 b	10 ± 1.1 ab
+N	122 ± 5 a	39 ± 2 c	8 ± 0.5 b	9 ± 1.3 b
+P	103 ± 6 a	31 ± 2 cd	10 ± 1.0 ab	15 ± 1.6 a

Values are the mean ± standard errors. For each type of soil, means followed by the same letters (a–d) are not significantly different at $P < 0.05$.

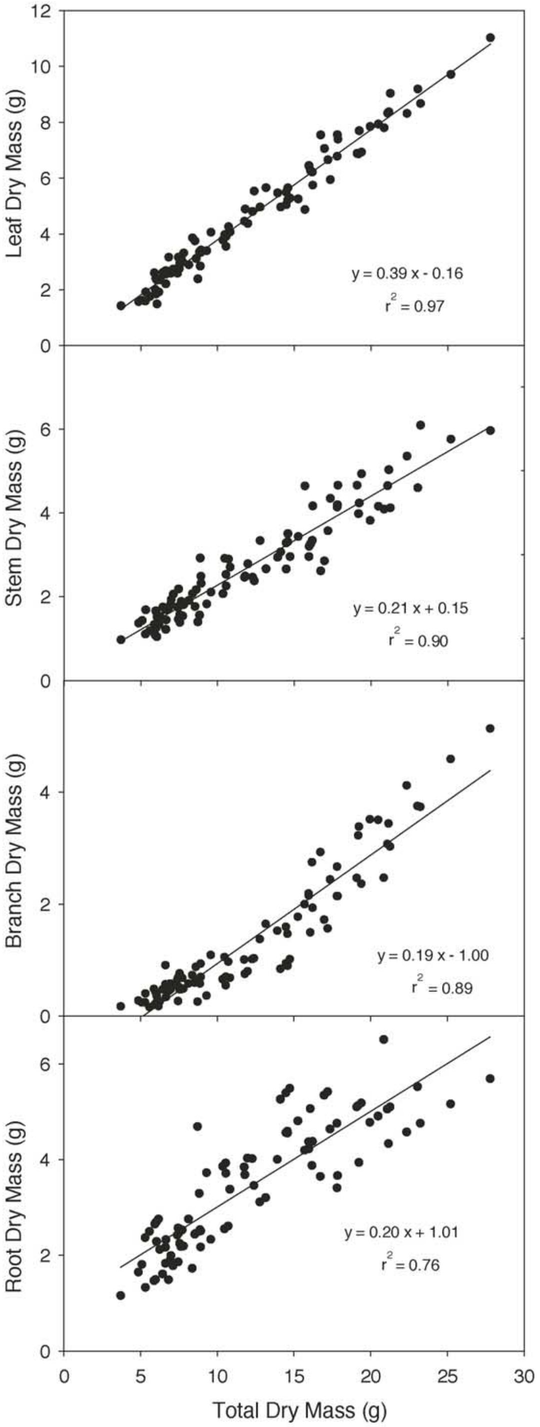

Fig. 2. Regression fits between total dry mass (g) and leaf, branch, stem and root dry mass (g) pooling all treatments (i.e., soil type, fertilization and water availability) together.

Table 4

Stomatal conductance (mmol H_2O m^{-2} s^{-1}) for the uppermost fully expanded leaves for non-fertilized (−F) and fertilized (+N and +P) treatments in two types of soil, with and without water stress

	Stomatal conductance (mmol m^{-2} s^{-1})	
	Well-watered	Stressed
Red sands		
−F	138 ± 16 ab	61 ± 14 bc
+N	262 ± 46 a	36 ± 11 c
+P	159 ± 27 ab	33 ± 9 c
Black soil		
−F	177 ± 22 a	19 ± 7 b
+N	191 ± 40 a	45 ± 12 b
+P	278 ± 59 a	44 ± 7 b

Values are means ± standard errors. For each type of soil, means followed by the same letters (a–c) are not significantly different at $P < 0.05$.

conductance in well-watered plants, but in water-stressed plants there was a trend (although not statistically significant) for fertilization to decrease stomatal conductance in the red sands, and to increase it in the black soil. The RWC of the leaves in which stomatal conductance was measured did not change with fertilization or stress treatment (data not shown).

In the red sands, osmotic potential at saturation (i.e., full turgor), osmotic potential at turgor loss point and RWC at turgor loss point decreased in the stressed treatments in non-fertilized plants, while in fertilized plants these parameters were hardly altered by stress (Table 5). Thus, there is evidence of osmotic adjustment in water-stressed, non-fertilized plants,

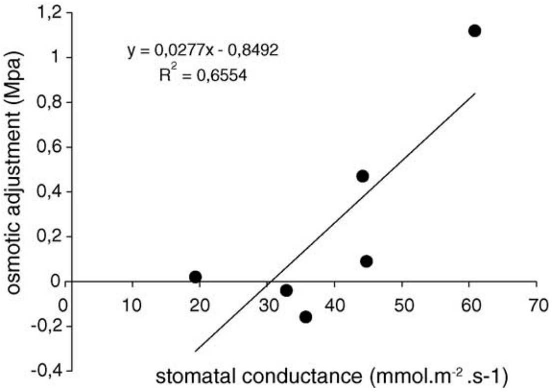

Fig. 3. Relationship between osmotic adjustment (i.e., the decrease in osmotic potential at full turgor) and stomatal conductance for the means of all treatments.

Table 5
Water relation parameters of shoots from non-fertilized (−F) and fertilized (+N and +P) plants in two types of soil, with and without water stress

	Osmotic potential at full turgor (MPa)		Osmotic potential at turgor loss point (MPa)		RWC at turgor loss point (%)	
	Well-watered	Stressed	Well-watered	Stressed	Well-watered	Stressed
Red sands						
−F	−1.27	−2.39	−1.45	−2.76	91.8	74.8
+N	−1.48	−1.32	−1.64	−1.74	89.1	83.3
+P	−1.37	−1.33	−1.70	−1.71	83.8	85.2
Black soil						
−F	−1.56	−1.58	−1.93	−2.00	82.1	83.8
+N	−1.26	−1.35	−1.61	−1.57	84.9	87.8
+P	−1.25	−1.72	−1.62	−2.16	83.9	78.5

Data derived from the analysis of pressure–volume curves. One curve for each treatment was adjusted ($P < 0.01$) with data from all the replications ($n = 6$) of each treatment.

but not in fertilized, water-stressed plants in the red sands. In the black soil, osmotic potential at saturation, osmotic potential at turgor loss point and RWC at turgor loss point did not change with stress in the non-fertilized and N treatments, while in P fertilized plants these parameters decreased with water stress, i.e., plants fertilized with P in the black soil adjusted osmotically. There was a strong correlation ($R^2 = 0.64$) between stomatal conductance and the degree of osmotic adjustment in water-stressed plants (Fig. 3).

4. Discussion

4.1. Fertilization and water availability effects on growth and water use efficiency

The moderate level of water stress applied reduced dry matter accumulation by about 50% in both types of soils. Drought periods lowering soil water potential to around −0.8 MPa are not uncommon under field conditions (Dye, 1996; O'Grady et al., 1999; Stoneman et al., 1996). Although the red sands and black soil are remarkably different in terms of texture and water-storing capacity, imposing a water stress of −0.8 MPa reduced growth to a similar extent in both soils. Fertilization was ineffective in promoting growth of water-stressed plants in the red sands, whereas in the black soil fertilization with P increased growth under water stress. This difference between soils may suggest that uptake of nutrients is more difficult at low water contents in the red sands than in the black soil. The uptake of nutrients depends on the demand by the plant and the ability of the soil to supply that demand (Nambiar and Sands, 1993). The rate of nutrient supply in the red sands may be low because soil hydraulic conductivity decreases more steeply at low water contents in sandy than in clayish soils (Arya et al., 1999). The lower availability of other nutrients in the red sands (Table 1) may also compromise any beneficial effects of fertilization with P or N in this type of soil.

In general, WUE was higher in the plants grown in the black soil than in the red sands, probably because of the higher availability of nutrients, and therefore, higher dry mass accumulation in the black soil. Water stress did not affect WUE. The effects of soil water deficit on WUE are varied, since photosynthesis, respiration and transpiration are involved and they can be affected to different extents depending on the environment (Sands and Mulligan, 1990). Differences in WUE between treatments were not due to differences in dry matter partitioning to the transpiring parts of the plants (i.e., the leaves, Fig. 2). Water availability in the black soil was greater than in the red sands, so it is conceivable that water stress developed more slowly in the black soil, allowing plants to adjust their water use to decreasing soil water availability (Choné et al., 2001). The lower WUE efficiency in the red sands may be due also to a higher energy cost to absorb nutrients from a very dilute soil solution. Xylem sap composition seems to be constant in spite of soil properties, so trees growing on poor soils are likely to allocate a larger proportion of energy to maintain a relatively constant xylem sap nutrient concentration at the cost of decreased growth (Smith and Shortle, 2001). In the red sands, root:total plant mass ratio was very high in non-fertilized plants, which reflects an increase in energy allocation to nutrient absorption. A decrease in dry matter allocation to roots with fertilization was also found in radiata pine, Douglas fir and loblolly pine seedlings (Sands and Mulligan, 1990). As reported for radiata pine seedlings (Sands and Mulligan, 1990), P fertilization increased WUE in well-watered plants in the red sands, probably because this treatment reduced allocation of C to root growth (Table 2).

4.2. Stomatal conductance and water relations

Stomatal conductance decreased with water deficit in both soils. A reduction of stomatal conductance by a decrease in soil water availability was reported in *Eucalyptus globulus* seedlings (Sasse and Sands, 1996), although in that experiment stomatal conductance of well-watered seedlings was 69.3 mmol $H_2O\ m^{-2}\ s^{-1}$, which is lower than the values we found in our well-watered plants (a mean for all well-watered treatments of 200 mmol $H_2O\ m^{-2}\ s^{-1}$), while stressed plants had similar values (around 40 mmol $H_2O\ m^{-2}\ s^{-1}$) in both studies. Transpiration in eucalypts is largely regulated by leaf area and air saturation deficit, but stomatal conductance is an important factor limiting transpiration (Whitehead and Beadle, 2004). Stomatal conductance in *E. grandis* is sensitive to changes in air water saturation deficit, and it is more sensitive in winter than in summer. The sensitivity of stomatal conductance to air saturation deficit affects the total amount of water transpired (Whitehead and Beadle, 2004). However, stomatal conductance is also altered by soil water deficit, as shown in Table 4, probably because stomatal conductance in this species depends on leaf water potential (Mielke et al., 1999) which drops under drought. Stomatal conductance declines with soil water deficit in some species of trees (i.e., *Liriodendron tulipifera* and *Pinus taeda*) but not in others, e.g., *Liquidambar styraciflua* and *Fraxinus americana* (Pataki and Oren, 2003). The mechanism that regulates stomatal response as the soil dries depends on an increase in the concentration of abscisic acid (Thomas and Eamus, 1999). Roots release more abscisic acid to the xylem as soil water availability decreases, with guard cells responding to the higher concentration of abscisic acid by closing stomata (Sauter et al., 2001).

Under water stress, there was a close correlation between the degree of osmotic adjustment (i.e., the difference in osmotic potential at full turgor between well-watered and water-stressed plants) and stomatal conductance. Likewise, there was a close correlation between osmotic adjustment and maintenance of stomatal conductance in water-stressed leaves of barley (González et al., 1999). Osmotic adjustment allows plants to extract water from drier soils (Dichio et al., 2003), and maintain a higher leaf turgor pressure (Kusaka et al., 2005). In chestnut oaks, fertilization with NPK enhanced osmotic adjustment in plants subjected to a drydown experiment for 10 weeks (Kleiner et al., 1992). In the red sands osmotic adjustment was observed only in non-fertilized plants. At any given value of RWC, osmotic adjustment increases leaf turgor pressure (González et al., 1999). The importance of osmotic adjustment conferring tolerance to water deficit is highlighted by the observation that osmotic adjustment decreased RWC at turgor loss point (i.e., wilting). Thus, fertilization impaired osmotic adjustment and increased RWC at turgor loss point in the red sands. Since RWC at midday was similar for all treatments, fertilized leaves (with no osmotic adjustment) were more likely than unfertilized ones (which adjusted osmotically) to be wilted for substantial periods of time throughout the day, which might offset any photosynthesis gains due to a better nutritional status. In fact, the percentage decrease of dry mass accumulation of water-stressed plants was lower for non-fertilized (55%) than for +N (63%) and +P (62%) plants in the red sands.

In the black soil only P fertilized plants showed osmotic adjustment in response to water deficit, and P fertilization clearly ameliorated growth under drought. In this soil, because of its physical and chemical properties, water depletion was slower and the soil solution is richer. Because of its greater water storage capacity, osmotic adjustment in the black soil may yield more water available to the plant, and might allow plants to maintain a better water status. In stressed plants in the black soil, the RWC at turgor loss point was lowest for plants fertilized with P, i.e., these were the plants less likely to be wilted at any point in time, which is consistent with the observation that these were the plants with the smallest growth reduction by water stress (63% for −F and +N compared to 57% for +P).

5. Conclusions

In conclusion, water stress tolerance is altered by fertilization, but the effect of fertilizers depends on soil properties. The same soil water potential produces different physiological results according to soil water availability and nutrient status. Osmotic adjustment confers tolerance to drought, and ameliorates growth under water deficit in soils with sufficient nutrients and high water storage capacity. In the red sands, fertilization with N or P reduced C allocation to roots and impaired osmotic adjustment, thereby causing a greater growth reduction under water stress. Regardless of soil type, fertilization with P produced the higher growth increases under well-watered conditions, and under water deficit conditions fertilization with P ameliorated growth in the black soil, in part due to osmotic adjustment. Therefore, fertilization with P is recommended in black soils, even if a period of moderate water stress is likely to occur. Fertilization with P in sandy soils, and with N in either soil type, is recommended only in good water availability conditions.

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