*Journal of Tropical Forest Science* 20(3): 205–210 (2008)

# EFFECTS OF FERTILIZATION AND WATER STRESS ON *EUCALYPTUS CAMALDULENSIS* SEEDLINGS

**M. T. Siddiqui***, **A. H. Shah & M. A. Tariq**

*Department of Forestry, University of Agriculture, Faisalabad, Pakistan*

*Received September 2007*

**SIDDIQUI, M. T., SHAH, A. H. & TARIQ, M. A. 2008. Effects of fertilization and water stress on *Eucalyptus camaldulensis* seedlings.** This study examined the responses of *Eucalyptus camaldulensis* seedlings to water stress and fertilization. To assess the effects of fertilization on osmotic adjustment under water stress conditions, 4-month-old *E. camaldulensis* plants were raised in pots and fertilized with either nitrogen (2 g of urea) or phosphorus (12 g of triple superphosphate). The soil was watered to attain one of the two conditions: field capacity and osmotic potential. Solute accumulation increased under the influence of P fertilizer in well-watered conditions, which indicates better performance under water stress. However, under water-stress conditions, control plants increased dry mass allocation to leaves but decreased allocation to roots. Water use efficiency, i.e. the grams of water transpired per gram of dry mass accumulated, was not altered by fertilization or water stress. N-fertilized plants increased allocation to roots and maintained allocation to leaves under water-stress conditions in comparison with control plants. They were not affected by water stress as much as P-fertilized plants. Osmotic adjustment was observed only in P-fertilized and control plants. Osmotic adjustment in water-stressed plants was associated with increased stomatal conductance. Water stress strategies were altered by fertilization and water availability.

Keywords: Growth response, osmotic adjustment, stomatal conductance, water use efficiency

**SIDDIQUI, M. T., SHAH, A. H. & TARIQ, M. A. 2008. Kesan pembajaan dan kekurangan air terhadap anak benih *Eucalyptus camaldulensis.*** Kajian ini menyiasat gerak balas anak benih *Eucalyptus camaldulensis* terhadap kekurangan air dan pembajaan. Bagi mengkaji kesan pembajaan terhadap pelarasan osmosis di bawah keadaan kurang air, *E. camaldulensis* berusia empat bulan ditanam di dalam pasu dan dibaja dengan nitrogen (2 g urea) atau fosforus (12 g superfosfat ganda tiga). Tanah disiram air untuk mencapai salah satu keadaan: muatan tanah atau potensi osmosis. Pengumpulan zat terlarut meningkat di bawah pengaruh baja P dalam keadaan air yang mencukupi. Ini menunjukkan prestasi yang lebih baik di bawah keadaan kekurangan air. Namun di bawah keadaan kekurangan air, anak benih kawalan menambah peruntukan jisim kering pada daun tetapi mengurangkan peruntukan pada akar. Kecekapan penggunaan air tidak dipengaruhi oleh pembajaan atau kekurangan air. Anak benih yang dibaja dengan N menambah peruntukan jisim kering ke akar tetapi mengekalkan peruntukan ke daun di bawah keadaan kekurangan air berbanding dengan kawalan. Anak benih tersebut tidak dipengaruhi oleh kekurangan air sebanyak mana kesan yang dialami oleh anak benih yang dibaja dengan P. Pelarasan osmosis hanya dicerap pada anak-anak benih yang dibaja dengan P dan kawalan sahaja. Pelarasan osmosis dalam anak benih yang mengalami kekurangan air berkait dengan aktiviti stoma yang bertambah. Strategi menangani kekurangan air dipengaruhi oleh pembajaan dan air.

## INTRODUCTION

Eucalypts are one of the most prominent tree species of Australia, covering almost 1000 species (Nicolle 2003). It grows in a wide range of climatic regions ranging from the tropics to temperate zone and from high rainfall to extreme drought (William & Brooker 1997). It was introduced in Pakistan in the 1960s. About 65% of Pakistan lies either in arid or semi-arid belt. To bridge the gap between wood demand and supply, eucalypts have been planted on a large scale on saline as well as dry soils in the country. *Eucalyptus* can withstand periods of soil and water deficit, and because of their deep root systems, they acclimatize to water shortage, typically suffering from non-lethal stress (Osorio *et al.* 1998). However, drought inhibits the

---

**E-mail: drmtahiruaf@hotmail.com*

growth severely. Water and nutrient status strongly influence the growth (plant dry mass partitioning, leaf area and assimilation rate) and physiology (photosynthesis) of *Eucalyptus* species (Sands *et al.* 1992, Whitehead & Beadle 2004). Susceptibility to nutrient stress can be reduced by traditional approaches such as fertilization (Burdett *et al.* 1984). Addition of N and P is reported to have significant effects on long-term increases in stand growth (Silfverberg 1996). Examining the interactions between water and nutrient availability is particularly important in understanding the responses of trees to each individual stress factor. Changes in biomass partitioning, leaf area and photosynthetic capacity per unit leaf area due to fertilizer application (Dye 1996) can also alter water-stress tolerance (Sperry *et al.* 2002, Graciono *et al.* 2006).

Fertilization of *Eucalyptus camaldulensis* is a standard practice in nursery and at transplanting in arid areas of Pakistan. However, there is no information on the effects of fertilization on water stress tolerance in young plants of this species. The objective of this study, therefore, was to determine the effects of fertilization with N and P on water relations and water stress in young *E. camaldulensis* plants.

## MATERIALS AND METHODS

Four-month-old *E. camaldulensis* plants were transplanted to earthen pots containing 4 kg of soil. Plants were fertilized with N and P at doses that were optimal for this soil based on previous fertilizer experiment (M. T. Siddiqui *et al.*, personal observation). N was applied at a rate of 2 g of urea (0.92 g of N) and P, 12 g of triple superphosphate (5.75 g of $P_2O_5$) per pot and mixed with soil. Ten control pots were established with no fertilizer. Fertilizers were applied at transplanting and the water-stress treatment began seven days after fertilization. For the drought treatment, 10 pots of each fertilizer treatment were watered to reach a mean soil water potential of -0.8 MPa throughout the experiment, while another set of 10 pots per fertilizer treatment was watered to field capacity (mean soil water potential -0.03 MPa) to serve as well-watered controls. Plants were grown under open nursery area conditions in the University of Agriculture, Faisalabad, Pakistan in 2006. To prevent evaporation from the soil, pots were placed in porous plastic bags which completely covered the soil surface. Pots were weighed every other day during the 20 days of water-stress treatment to estimate daily transpiration. After weighing, pots were watered to their target soil water contents. Well-watered plants were watered every other day to reach field capacity. The water-stressed plants were only watered when they reached -0.8 MPa, i.e. 4.15% of gravimetric water content. Soil water potential was determined with the pressure-plate method at 0, 0.05, 0.1, 0.3 and 0.8 MPa of balance pressure (Richards *et al.* 1956).

The mean transpiration rate was determined from the change in pot mass between successive measurements every day. The transpiration rate was calculated on a leaf-mass basis by dividing the mean daily amount of water transpired between day 15 and 20 of the experiment by final leaf mass on day 20.

Plant dry mass was determined at the beginning of the experiment by randomly taking a sample of 20 plants. At the end of the experiment, all the plants of each treatment (n=10) were also weighed dry. Dry mass was obtained after drying the plant material in an oven at 70 °C until constant weight. The increase in dry weight was calculated by subtracting final total dry weight from the mean initial dry weight. Plants were divided into leaves, branches, stem and roots to determine dry-matter partitioning.

At the end of the experiment, six plants per treatment were randomly chosen for measurements of stomatal conductance. Stomatal conductance of the uppermost fully expanded leaf was measured at midday under sunny skies with a steady state porometer. Pressure–volume curves were fitted by measuring relative water content (RWC) at different values of water potential on six terminal branches per treatment (Tyree & Richter 1982). Twigs were cut at dawn. Measurements were taken after twigs were kept for two hours in black plastic bags with their ends in water to reach full turgor. Therefore, branches were allowed to transpire freely, and water potential and fresh mass were measured seven times during this dehydration period to provide a range of RWC values adequate for deriving pressure–volume curves. The water potential of terminal twigs was measured before dawn and at midday 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.

Thereafter, plant material were oven dried to constant mass in order to calculate RWC. RWC was calculated as:

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight}) \times 100}{(\text{Full turgor fresh weight} - \text{Dry weight})}$$

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

### Statistical analysis

Data were analyzed by Mstat with fertilizer treatments and water availability as main factors. Means differences were compared using Tukey's test ($p < 0.05$).

## RESULTS AND DISCUSSION

Potting medium consisted of clay loam soils having a pH of 7.2. Physical and chemical characteristics of potting medium are given in Table 1. Fertilization with P increased dry matter accumulation in well-watered plants, but none of the fertilizers had a growth promoting effect on water-stressed plants (Table 2). P had a greater effect than N on dry-mass accumulation in well watered plants. Absolute dry-mass allocation to leaves (g dry mass) was greater with P applications under both water conditions. However, N fertilization did not increase allocation to leaves in any treatment. The stem mass / total plant mass ratio in control plants was lower when they were well-watered compared with when they were under water stress. N decreased the stem / total plant mass ratio in plants under water stress (Table 2). The root mass / total plant mass ratio was not affected by water stress in control plants, whereas in both fertilization treatments, the root mass / total plant mass ratios were higher in stressed plants than in well-watered plants. In water-stressed plants, N increased allocation to roots compared with control plants. On the other hand, P fertilization decreased allocation to roots under both water availability conditions, and plants fertilized with P showed the lowest root mass / total plant mass ratio.

The mean daily transpiration rate was lower in water-stressed plants compared with well-watered ones (Table 3). When plants were well watered, the transpiration rate was higher in fertilized than in control plants. Fertilization had no effect on the mean daily transpiration rate of stressed plants. Water loss on a leaf-mass basis was higher in the control and plants fertilized with N in well-watered conditions than under water stress (Table 3). On the other hand, plants fertilized with P have the same transpiration rate on a leaf-mass basis under both water conditions.

Relative water content at turgor-loss point was higher in stressed than in well-watered plants, but was not affected by fertilization (Table 4). In the case of control and N-fertilized plants, osmotic potential at turgor-loss point and osmotic potential at full turgor were higher in well-watered than in stressed plants. However, in P-fertilized plants, osmotic potential was not altogether affected by water stress.

Addition of P increased dry-mass accumulation in well-watered plants, but the growth promoting effects of both N and P fertilizers were suppressed by water stress (Table 2). Fertilization is more effective under well-watered conditions in

**Table 1** Physical and chemical properties of soil used in the experiment

| | |
|---|---|
| pH | 7.4 |
| Electrical conductivity(dS $m^{-1}$) | 0.06 |
| Total N (%) | 0.02 |
| P (ppm) | 4.72 |
| Ca (mequiv./100 g) | 2.1 |
| Mg (mequiv./100 g) | 0.82 |
| Na (mequiv./100 g) | 0.52 |
| K (mequiv./100 g) | 0.33 |
| C (%) | 0.38 |
| CEC (mequiv./100 g) | 8.22 |
| Clay (%) | 10.3 |
| Silt (%) | 9 |
| Sand (%) | 82.3 |
| Soil texture | Sandy loam |

Standard methods were used to analyze the soil, e.g. pH in water 1:2.5 w/v, EC in soil paste, N by micro-Kjeldhal method (Jackson 1962), P by Kurtz and Bray method (1945), C by Walkley and Black method (1934), and Ca, Mg, Na, K and CEC in ammonium acetate (pH 7) by atomic absorption. Soil texture was determined by the methods of Boyocous (1962).

**Table 2** Increase in total dry mass, and ratios of branch mass, leaf mass, stem mass and root mass to total plant mass for control and N- and P-fertilized plants, with or without water stress, in 4-month-old *Eucalyptus camaldulensis*

| | Increase in total dry mass (g) | Branch mass/ total mass | Leaf mass/ total mass | Stem mass/ total mass | Root mass/ total mass |
|---|---|---|---|---|---|
| Well-watered plants | | | | | |
| Control | 8.95 (0.61) b | 0.06 (0.01) c | 0.42 (0.01) b | 0.20 (0.01) c | 0.30 (0.01) c |
| N | 11.60 (0.60) ab | 0.10 (0.01) b | 0.39 (0.01) bc | 0.20 (0.01) c | 0.31 (0.01) b |
| P | 18.62 (3.21) a | 0.15 (0.01) a | 0.48 (0.01) a | 0.24 (0.01) abc | 0.19 (0.01) d |
| Water-stressed plants | | | | | |
| Control | 6.52 (0.54) c | 0.08 (0.01) c | 0.38 (0.01) c | 0.29 (0.01) a | 0.33 (0.01) b |
| N | 6.34 (0.24) c | 0.20 (0.01) c | 0.39 (0.01) c | 0.25 (0.01) bc | 0.38 (0.01) a |
| P | 4.23 (0.22) c | 0.08 (0.01) | 0.45 (0.02) a | 0.27 (0.01) ab | 0.24 (0.01) c |

Values are means. Standard errors in parentheses. Values followed by the same letter are not significantly different at $p < 0.05$.

**Table 3** Mean daily transpiration rates and transpiration rates on a leaf-mass basis for control and N- and P-fertilized 4-month-old *Eucalyptus camaldulensis*, with and without water stress

| | Mean daily transpiration rate (g water. plant$^{-1}$. day$^{-1}$) | Transpiration rate on a leaf-mass basis (g water.g leaf dry mass$^{-1}$. day$^{-1}$) |
|---|---|---|
| Well-watered plants | | |
| Control | 59 (4) b | 30 (2) a |
| N | 86 (4) a | 31 (1) a |
| P | 100 (13) a | 26 (3) ab |
| Water-stressed plants | | |
| Control | 19 (2) c | 19 (3) bc |
| N | 25 (2) c | 12 (1) c |
| P | 23 (2) c | 29 (6) ab |

Values are means. Standard errors in parentheses. Values followed by the same letter are not significantly different at $p < 0.05$.

other species of the eucalypts such as *E. grandis* (Stoneman *et al.* 1996, Graciano *et al.* 2006), which have few of the drought resistance traits among other eucalypt species (Specht 1996). The present study revealed a similar response (i.e. growth increase by P in well-watered plants but not under drought). Our results are in conformity with Graciano *et al.* (2005). Sicard *et al.* (2006) reported an increase in leaf biomass in Norway spruce and Douglas-fir plantations due to initial fertilization. Similar N effects have been reported by Xue *et al.* (2004) in Douglas-fir provenances.

Fertilization and water availability altered dry-mass partitioning to roots and leaves. In general, dry-mass partitioning to roots increases in water-stressed plants in order to explore and take up water from a larger rooting zone (Sperry *et al.* 2002, Ludovici & Kress 2006). Total root mass / total plant mass was lower in P-fertilized plants than in control and N-fertilized plants (Table 2). It is evident that P fertilization of plants in this type of soil increases the leaf contents of N and P (Searson *et al.* 2004, Warren *et al.* 2005). Therefore, the overall nutritional status of these plants is improved by P-fertilization, and the decrease in the root mass / total plant mass ratio is consistent with numerous studies that show low root mass ratios in plants growing in high nutrient conditions (Kriedemann &

**Table 4** Leaf water relations of shoots from control and N- and P-fertilized 4-month-old *Eucalyptus camaldulensis* plants, with and without water stress.

| | Well-watered plant | Water-stressed plant |
|---|---|---|
| Relative water content at turgor-loss point (%) | | |
| Control | 85 | 90 |
| N | 85 | 91 |
| P | 85 | 91 |
| Osmotic potential at turgor-loss point (MPa) | | |
| Control | -1.8 | -3.09 |
| N | -1.78 | -1.99 |
| P | -1.84 | -1.8 |
| Osmotic potential at full turgor (MPa) | | |
| Control | -1.46 | -1.89 |
| N | -1.42 | -1.68 |
| P | -1.64 | -1.64 |

Data were derived from analysis of pressure–volume curves. One curve for each treatment was adjusted using data from all the replications (n = 6) of each treatment.

**Table 5** Stomatal conductance (mmol $H_2O$ $m^{-2}$ $s^{-1}$) for the uppermost fully expanded leaves for control and fertilized (N and P) treatments, with and without water stress

| Treatment | Well-watered | Stressed |
|---|---|---|
| Control | 173 (20) a | 17 (6) b |
| N | 187 (37) a | 42 (10) b |
| P | 275 (57) a | 41 (7) b |

Values are means and standard errors in parentheses. For each type of soil, means followed by the same letters are not significantly different at $p < 0.05$

Cromer 1996, Graciano *et al.* 2006). Thus, the proportion of dry mass allocated to roots depends on both nutrient and water availability. P fertilization reduced dry-mass allocation to roots and this reduction was not reversed under water-stress conditions, although plants were small. Therefore it might be due to lower soil water exploration that under drought P-fertilized plants grew less than control plants.

The transpiration rate was higher in well-watered than in water-stressed plants (Table 3). Osmotic adjustment, i.e. the accumulation of compatible solutes in response to the decrease in water potential of the cell's environment (Monteith & Unsworth 1990, Babu *et al.* 1999), allows plants to avoid wilting of the younger tissues and thereby sustain leaf expansion. Control plants adjusted osmotically by reducing the osmotic potential at full turgor by more than 0.4 MPa.

Mean daily transpiration rate was the same for all water-stressed treatments (Table 4), so soil water depletion was the same in all stressed treatments. Therefore, osmotic adjustment allowed leaves of control and N-fertilized plants to maintain turgor better than those of P-fertilized plants. This may have resulted in higher growth rate with the same amount of water transpiration (Table 4). Transpiration on a leaf mass basis decreased with water stress in control and N-fertilized plants but not in P-fertilized plants. Stomatal conductance was four to seven folds lower in stressed than in well-watered plants (Table 5). Fertilization did not change stomatal conductance in well-watered plants, but in water-stressed plants there was a trend (although not statistically significant). Thus P-fertilization seems to impair the capacity to reduce water use under water deficit, probably because P-fertilized plants down regulated stomatal conductance less than control and N-fertilized plants under water stress.

## CONCLUSIONS

Water stress tolerance is affected by the application of fertilizers. Soil water potential produces different physiological attributes according to water availability and nutrient status. P fertilization in well-watered plants decreased osmotic potential. However, under water stress conditions, unfertilized plants showed osmotic adjustment while P-fertilized did not. Osmotic adjustment confers tolerance to water stress, and ameliorates growth in soils with sufficient nutrients and high water storage capacity. Regarding managemet practices, fertilization during planting produces changes in water relations (and presumably drought tolerance) that continue for at least one year and fertilization with P may increase drought susceptibility in young *E. camaldulensis* plants.

## REFRENCES

Babu, R. C., Pathan, M. S., Blum, A. & Nguyen, H. T. 1999. Comparison of measurement methods of osmotic adjustment in rice cultivars. *Crop Science* 39: 150–158.

Boyocous, G. J. 1962. Hydrometer method improved for making particle-size analysis of soils. *Agronomy Journal* 54: 464–465.

Burdett, A. N., Herring, L. J. & Thompson, C. F. 1984. Early growth of planted spruce. *Canadian Journal of Forest Research* 14: 644–651.

Cochard, H., Forestier, S. & Ameglio, T. 2001. A new validation of the Scholander pressure chamber technique based on stem diameter variations. *Journal of Experimental Botany* 52: 1361–1365.

Dye, P. J. 1996. Response of *Eucalyptus grandis* trees to soil water deficits. *Tree Physiology* 16: 233–238.

Graciano, C., Guiamet, J. J. & Goya, J. F. 2005. Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. *Forest Ecology Management* 212: 40–49.

Graciano, C., Guiamet, J. J. & Goya, J. F. 2006. Fertilization and water stress interactions in young *Eucalyptus grandis* plants. *Canadian Journal of Forest Research* 36: 1028–1034.

Jackson, M. L. 1962. *Soil and Chemical Analysis.* Printce Hall, New Jersey.

Kriedemann, P. E. & Cromer, R. N. 1996. The nutritional physiology of the eucalypts—nutrition and growth. Pp. 109–121 in Adams, M. A.(Eds.) *Nutrition of Eucalypts.* CSIRO Publishing, Collingwood.

Kurtz, I. T. & Bray, R. H. 1945. Determination of total organic and available forms of phosphorus in soil. *Soil Science* 59: 39–45.

Ludovici, K. H. & Kress, L. W. 2006. Decomposition and nutrient release from fresh and dried pine roots under two fertilizer regimes. *Canadian Journal of Forest Research* 36: 105–111.

Monteith, J. L. & Unsworth, M. H. 1990. *Principles of Environmental Physics.* Second edition. Edward Arnald, London.

Nicolle, D. 2003. *Currency Creek Arboretum (CCA) Eucalyptus Research, Vol. 2.* Available at http://www.dn/com.au/

Osorio, J., Osrio, M. L. & Chaves, M. M. 1998. Water deficits are more important in delaying growth than in changing patterns of carbon allocation in *Eucalyptus globulus. Tree Physiology* 18: 363–373.

Richards, L., Gardner, W. & Ogata, G. 1956. Physical process determining water loss from soil. *Soil Science Society of American Proceedings* 20: 310–314.

Sands, P. J., Cromer, R. N. & Kirschbaum, M. U. F. 1992. A model of nutrient response in *Eucalyptus grandis* seedlings. *Australian Journal of Plant Physiology* 19: 459–470.

Searson, M. J., Thomas, D. S., Monyagu, K. D. & Conroy, J. P. 2004. Leaf water use efficiency differs between *Eucalyptus* seedlings from contrasting rainfall environment environments. *Functional Plant Biology* 31: 441–450.

Schulte, P. J. 1998. *Pressure–Volume Curve Analysis Program. User's Guide.* University of Nevada, Las Vegas.

Sicard, C., Saint-Andre, L. & Ranger, D. G. 2006. Effect of initial fertilization on biomass and nutrient content of Norway spruce and Douglas-fir plantations at the same site. *Trees* 20: 229–246.

Silfverberg, K. 1996. *Nutrient Status and Development of Tree Stands and Vegetation on Ash-Fertilized Drained Peatlands in Finland.* Research Papers 588. The Finnish Forest Research Institute, Helsinki.

Sperry, J. S., Hacke, U. G., Oren, R. & Comstock, J. P. 2002. Water deficits and hydraulic limits to leaf water supply. *Plant Cell and Environment* 25: 251–263.

Specht, R. L. 1996. The influence of soils on the evolution of eucalypts. Pp. 31–60 in Adam, M. (Ed.) *Nutrition of Eucalypts.* CSIRO Publishing, Collingwood.

Stoneman, G. L., Crombie, D. S., Whiteford, F. J., Giles, R., Portlock, C. C., Galbraith, J. H. & Dimmock, G. M. 1996. Growth and water relations of *Eucalyptus marginata* (jarrah) stands in response to thinning and fertilization. *Tree Physiology* 16: 267–274.

Tyree, M. T. & Richter, H. 1982. Alternate methods of analyzing water potential isotherms: some cautions and clarifications. II. Curvilinearity in water potential isotherms. *Canadian Journal of Botany* 60: 911–916.

Walkley, A. & Black, I. A. 1934. An examination of the Degtjareft Method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* 37: 29–38.

Warren, C. R., Tausz, M. & Adams, M. A. 2005. Does rainfall explain variation in leaf morphology and physiology among populations of red ironbark (*Eucalyptus siderelon* subspp. *Tricarpa*) growing in a common garden? *Tree Physiology* 25: 1369–1378.

Whitehead, D. & Beadle, C. L. 2004. Physiological regulations in productivity and water use in *Eucalyptus*: a review. *Forest Ecology Management* 193: 113–140.

William, J. E. & Brooker, M. I. H. 1997. Eucalypts: an introduction. Pp. 1–15 in William, J. E. & Woinarski, C. Z. (Eds.) *Eucalypt Ecology.* Cambridge University Press, Cambridge.

Xue, J., Sands, R. & Clinton, P. W. 2004. Growth and physiological responses of two Douglas-fir provenances to nitrogen supply. *New Zealand Journal of Forest Science* 34: 59–76.