PRODUCTION OF *EUCALYPTUS CLOEZIANA* CUTTINGS IN RESPONSE TO STOCK PLANT TEMPERATURE

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TRUEMAN SJ, MCMAHON TV & BRISTOW M. 2013. Production of *Eucalyptus cloeziana* cuttings in response to stock plant temperature. Propagation of tropical and subtropical eucalypts is often limited by reduced production of rooted cuttings in winter. We assessed whether changing the temperature of stock plants of *Eucalyptus cloeziana* from 28/23 (day/night) to 18/13, 23/18 or 33/28 °C affected the production, nutrient concentrations and percentages of cuttings that subsequently formed roots. Lowering the temperature to 18/13 or 23/18 °C greatly reduced the number of cuttings harvested from stock plants but did not affect the percentage of cuttings that formed roots. However, raising the temperature to 33/28 °C greatly increased the number of cuttings produced by stock plants and the ensuing percentage of cuttings that formed adventitious roots, thereby increasing the final number of rooted cuttings produced from each stock plant. The effects of stock plant temperatures on rooting were not the result of altered nutrient concentrations. However, consistent relationships were found between adventitious root formation and boron concentration. Rooting percentages were very low (1–14%) but rooted cutting production per stock plant (e.g. 12 rooted cuttings over a 14-week period at 33/28 °C) was sufficient to establish field tests for clonal plantation forestry.

Keywords: Adventitious roots, auxin, boron, calcium, Myrtaceae, propagation

TRUEMAN SJ, MCMAHON TV & BRISTOW M. 2013. Kesan suhu stok tanaman terhadap penghasilan keratan *Eucalyptus cloeziana*. Pembiakan *Eucalyptus* di kawasan tropika dan subtropika lazimnya dihadkan oleh kekurangan penghasilan keratan berakar pada musim sejuk. Kami mengkaji sama ada perubahan suhu stok tanaman *Eucalyptus cloeziana* daripada 28 °C/23 °C (siang/malam) kepada 18 °C/13 °C, 23 °C/18 °C atau 33 °C/28 °C boleh mempengaruhi penghasilan keratan, kepekatan nutrien dan peratusan keratan yang kemudiannya membentuk akar. Penurunan suhu kepada 18 °C/13 °C atau 23 °C/18 °C mengurangkan dengan banyaknya bilangan keratan daripada stok tanaman tetapi ia tidak mempengaruhi peratusan keratan yang membentuk akar. Bagaimanapun, peningkatan suhu kepada 33 °C/28 °C menambah dengan banyaknya bilangan keratan yang dihasilkan daripada stok tanaman dan peratusan keratan yang kemudiannya membentuk akar adventitius. Seterusnya, ini meningkatkan jumlah akhir keratan berakar yang dihasilkan daripada setiap stok tanaman. Namun, hubungan yang konsisten diperhatikan antara pembentukan akar adventitius dengan kepekatan boron. Peratusan pengakaran adalah sangat rendah (1%–14%) tetapi penghasilan keratan berakar setiap stok tanaman (iaitu 12 keratan akar selama tempoh 14 minggu pada 33 °C/28 °C) adalah mencukupi untuk mengasaskan ujian lapangan bagi perhutanan tani klon.

INTRODUCTION

Clonal selection and propagation of cuttings are commonly used methods in horticulture and forestry to improve yield and product uniformity. Propagation of cuttings is also used when seedling supply is limited by sporadic flowering, low seed production, poor germination or high cost of producing hybrid seed (Pohio et al. 2005, Dickinson et al. 2010, Pijut et al. 2011). Some of the world's most widely grown eucalypts such as *Eucalyptus camaldulensis, E. grandis* and *E. urophylla* are produced from cuttings (Wendling & Xavier 2005, Cunha et al. 2009a, b, Wu et al. 2011). However, most plantation eucalypts are considered difficult to propagate from cuttings (Assis et al. 2004, Smith & Henson

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2007, Kilkenny et al. 2012). This includes one of the most valuable species for tropical and subtropical regions, *Eucalyptus cloeziana* (Assis et al. 2004, Almeida et al. 2007). This species is grown from seedlings in many countries including Brazil, China and Australia for high-quality timber products (Chen et al. 2010, Redman & McGavin 2010).

Difficulties with vegetative propagation of woody plants are overcome once stock plant management, propagation environment and post-severance treatments have been optimised (Leakey 2004, Atangana et al. 2006, Trueman et al. 2007, Wendling et al. 2010). A major limitation in eucalypt propagation in the subtropics is a decline in the production of rooted cuttings in winter. This decline has been attributed to reduced calcium (Ca) uptake into shoots of stock plants at low temperature (Assis et al. 2004). Subsequent research has found that, while low temperatures may reduce the number of cuttings produced from stock plants, they have variable effects on the ensuing percentage of cuttings that form roots (Trueman & Richardson 2008, Cunha et al. 2009b, Trueman et al. 2013). Two of these studies of *Corymbia torelliana* × *C. citriodora, E. grandis, E. urophylla* and *E. grandis* × *E. urophylla* monitored ambient temperatures across seasons and did not separate temperature effects during shoot production by stock plants from temperature effects in the subsequent phase of root formation by the harvested cuttings (Trueman & Richardson 2008, Cunha et al. 2009b). One study, however, showed that stock plant temperature primarily regulated shoot production rather than subsequent root formation in *C. citriodora*, whereas it strongly affected both shoot production and root formation in *E. dunnii* (Trueman et al. 2013). In that study, lowering the stock plant temperature did not affect the Ca concentration of cuttings, but root formation was consistently associated with boron (B) concentration (Trueman et al. 2013). Production of rooted cuttings during winter in temperate regions usually occurs in custom-built propagation structures that provide a highly protected microclimate for cuttings (Hartmann et al. 1997). These facilities often provide temperature control or supplemental lighting to improve survival of cuttings, promote root formation and accelerate growth of rooted cuttings (Majada et al. 2011, Pijut et al. 2011, Currey et al. 2012, Martínez-Alonso et al. 2012). Eucalypt nurseries in the tropics and subtropics generally provide only partially-protected environments such as mist-irrigated polyethylene chambers or greenhouses for cuttings after they have been severed from stock plants (McNabb et al. 2002, Assis et al. 2004, Saya et al. 2008). Climatic control for stock plants is often much less than that provided for cuttings.

In this study, we determined the response of *E. cloeziana* stock plants to changing temperature. We hypothesised that changing the temperature would affect the production of cuttings by stock plants, the Ca or B concentration of cuttings and the percentage of cuttings that formed roots. Specifically, we assessed the: (1) weight and number of cuttings produced by stock plants at four different temperatures, (2) concentrations of Ca, B and other nutrients in cuttings at four temperatures, (3) subsequent root formation and cumulative production of rooted cuttings, and (4) relationships between Ca, B as well as other nutrient concentrations and the percentages of cuttings that formed roots. These results will assist in developing clonal propagation systems for *E. cloeziana* and other tropical and subtropical eucalypts.

MATERIALS AND METHODS

Stock plants

We obtained seeds of *E. cloeziana* (Wolvi State Forest; 26° 7' S, 152° 46' E) from the Australian Tree Seed Centre. Seeds were sown in January 2009 in potting mix consisting of a 75/25 (v/v) mixture of shredded pine bark and perlite with 3 kg of 8- to 9-month slow release Osmocote™ fertiliser, 3 kg of lime, 1 kg of gypsum, 1 kg of Micromax™ granular micronutrients and 1 kg of Hydroflo™ soil wetting agent incorporated per m^3. Seeds were covered with a thin layer of vermiculite and germinated under mist irrigation in a glasshouse in Gympie (26° 11' S, 152° 40' E). Misting was provided for 10 s every 10 min from 0600 to 1800 hours and for 10 s every 20 min from 1800 to 0600 hours.

We transplanted the seedlings in February 2009 into 2.8 L pots filled with the same potting mix (described above) and then transferred them randomly into four controlled temperature glasshouse chambers in Nambour (26° 38' S, 152° 56' E). There were 14 seedlings in each chamber. Temperatures in all chambers were set at 28/23 °C (day/night; 0600–1800 hours/1800–0600 hours respectively). Water for initial seedling establishment was provided by trickle irrigation for 1 min every hour from 0800 till 1700 hours, for 1 min at 2200 hours, and for 1 min at 0400 hours. After 4 weeks, we reduced the trickle irrigation to 1 min every 3 hours from 0800 till 1700 hours, 1 min at 2200 hours and 1 min at 0400 hours. Commencing in April 2009, we managed the seedlings as stock plants by pruning at 3-week intervals to a height of ~30 cm and a canopy diameter of ~20 cm. The last pruning before the start of the experimental treatments was performed on 8 June 2009.

Experimental design

We changed the temperatures in three of the chambers on 15 June 2009 to provide four temperature treatments across the four chambers: 18/13, 23/18, 28/23 and 33/28 °C (day/night, as described above). These treatments were based on a study of temperature responses in seedlings of *E. cloeziana* and *E. argophloia* (Ngugi et al. 2003). The four treatments were termed 18, 23, 28 and 33 °C respectively. Treatments were allocated randomly to chambers. To minimise effects of chamber, temperatures and their corresponding stock plants were randomly relocated to a different chamber every 4 weeks. To minimise the effect of light gradients within chambers, stock plant positions within chambers were also randomised periodically.

All available cuttings of all stock plants were harvested at 2, 5, 8, 11 and 14 weeks after commencement of the four temperature treatments. The total fresh weight and number of cuttings were recorded for each stock plant on each occasion. A random sample of nine cuttings per stock plant (or all cuttings, if less than nine were available) was then prepared on each occasion by trimming cuttings to approximately 5-cm length and removing half to two-thirds of the length of each leaf. Cuttings possessed two leaves because leaves were opposite in the juvenile-phase shoots of *E. cloeziana*. Cuttings were placed, without auxin treatment, 1 cm deep into a 12-cm^3 tube (Hung & Trueman 2010, 2011) containing a 75/25 (v/v) mixture of perlite and shredded pine bark with 3 kg of 8- to 9-month slow-release Osmocote™ fertiliser and 1 kg of gypsum incorporated per m^3. Cuttings from additional stock plants were treated with auxin (3 g indole-3-butyric acid/kg powder) but these rooted very poorly and so their results were not presented.

Trays of cuttings were placed in the same glasshouse where seeds were germinated (see above), with mist irrigation provided for 10 s every 15 min from 0600 till 1800 hours and 10 s every 20 min from 1800 till 0600 hours. Trays were placed on TPS080 heated root-beds to maintain ~25 °C at the base of each tray for 4 weeks and then moved to ambient glasshouse condition for a further 5 weeks. Irradiances and temperatures during the experiment have been reported previously (Trueman et al. 2013). Cuttings were carefully removed from the propagation medium after 9 weeks and the proportion of cuttings forming roots was recorded for each stock plant.

Nutrient analyses

All cuttings of three stock plants per temperature were harvested at 2, 8 and 14 weeks after commencement of the four temperature treatments. Different stock plants were harvested on each occasion. The cuttings were placed in a paper bag, dried for 7 days at 65 °C, weighed and then ground using a tissue homogeniser. The concentrations of N and S were determined by combustion analysis (McGeehan & Naylor 1988, Rayment & Higginson 1992). The concentrations of P, K, Al, B, Ca, Fe, Mg, Mn, Na and Zn were determined by inductively-coupled plasma-atomic emission spectroscopy (Munter & Grande 1981) after nitric and perchloric acid digestion (Martinie & Schilt 1976).

Statistical analyses

Cumulative fresh weight, cumulative number of cuttings, proportions of cuttings with roots and cumulative number of rooted cuttings per stock plant were analysed by one-way analysis of variance (ANOVA), comparing four temperatures. Differences in proportions of cuttings forming roots between the five harvest dates within each temperature were also analysed by one-way ANOVA. Nutrient concentrations were analysed by one-way ANOVA, comparing four temperatures within each harvest date or comparing three harvest dates within each temperature because significant temperature × harvest date interactions were detected by two-way ANOVA. Post-hoc least significant difference tests were performed when significant differences were detected by ANOVA. Weights or numbers were square root or log transformed and proportions were arcsine square root transformed when variance was heterogeneous. One-tailed linear regressions with mean nutrient concentration and rooting percentage as the independent and dependent variables respectively and correlations between the concentrations of each of the nutrients were also calculated. Means were reported with standard errors and treatment differences or interactions were regarded as significant at $p < 0.05$.

RESULTS

Stock plants of *E. cloeziana* produced the highest weight of cuttings at 33 and 28 °C (Figure 1a) and the highest number of cuttings at 33 °C (Figure 1b). The lowest weight and lowest number of cuttings were obtained at 18 and 23 °C (Figures 1a and 1b). Final numbers of cuttings per stock plant ranged from 35 ± 3 to 102 ± 10.

The percentage of cuttings that subsequently formed roots was low and stock plant temperature did not significantly affect rooting percentages within four of the five individual harvest dates (Figure 2a). However, cuttings from stock plants at 33 °C provided higher rooting than cuttings from other temperatures on the first harvest date (i.e. 2 weeks) and also when rooting percentages for each stock plant were averaged across all five harvest dates (Figure 2a). Average rooting percentages ranged from 1 ± 1% to 14 ± 3%. Final production of rooted cuttings was much higher when stock plants were grown at 33 °C (Figure 2b). Final numbers of rooted cuttings per stock plant ranged from 2 ± 1 to 12 ± 3.

Stock plant temperature generally did not affect nutrient concentrations of cuttings and nutrient concentrations only declined from 2 to 14 weeks on 2 out of the 48 possible occasions (12 nutrients × 4 temperatures)—specifically N and P at 33 °C. Therefore, N, P, K, Al, Fe, Mg, Mn, Na, S and Zn concentrations were not presented. Stock plant temperature did not significantly affect the Ca concentration of cuttings (Figure 3a). The percentage of cuttings that formed roots was not significantly related to Ca concentration (Figure 3b). However, rooting percentages were positively related to B concentration (Figure 4b), even though stock plant temperature had no effect on B concentration (Figure 4a). The concentrations of many nutrients were correlated with one another, including B with N, P, K, Ca, Fe, Mg, Mn and S (Table 1). Despite these correlations, rooting percentages were not significantly related to the concentrations of other nutrients except for N, P and K (Table 2).

DISCUSSION

High temperature (33 vs 28, 23 or 18 °C) greatly increased the number of cuttings produced by *E. cloeziana* stock plants, the ensuing percentage of cuttings that formed adventitious roots and, therefore, the final number of rooted cuttings produced from each stock plant. The optimal temperatures for shoot biomass production were 33 and 28 °C, consistent with optimal temperatures for light-saturated photosynthesis in seedlings of *E. cloeziana* and another subtropical eucalypt, *E. argophloia* (Ngugi et al. 2003). Cuttings of *E. cloeziana* proved very difficult to root under the subtropical winter and spring conditions of the glasshouse, with average rooting percentages (1–14%) similar to those found previously (10%) at the same location in

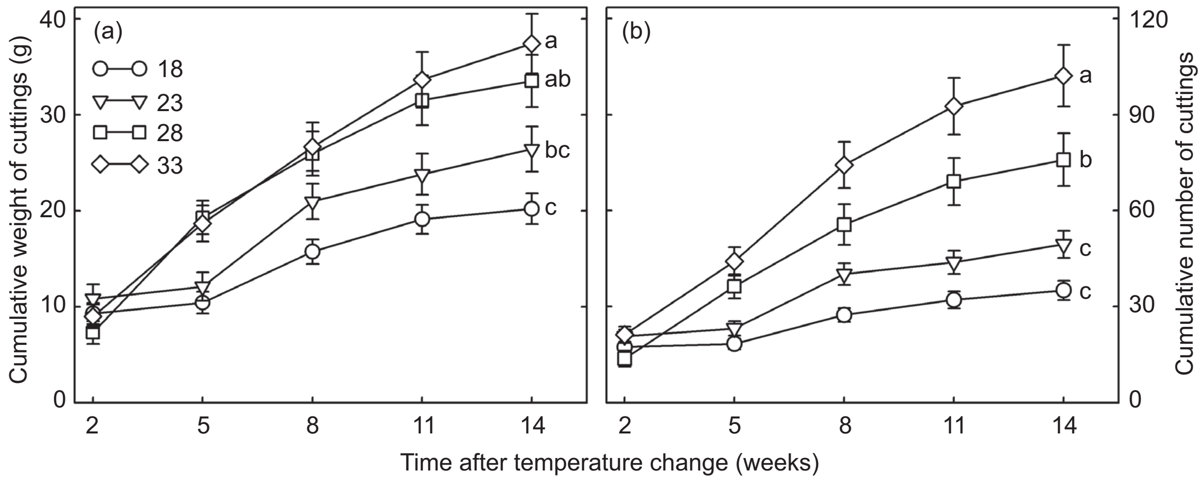

Figure 1 (a) Cumulative weight and (b) cumulative number of cuttings produced per *Eucalyptus cloeziana* stock plant after temperature was changed from 28 to 18, 23 or 33 °C; final means (± SE) with different letters are significantly different (ANOVA and LSD test, $p < 0.05$, n = 14)

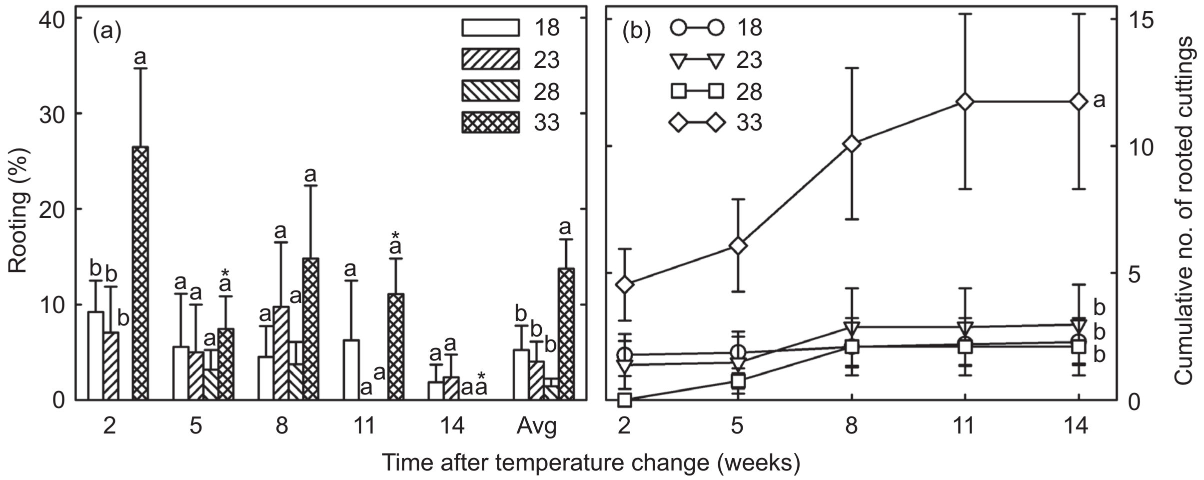

Figure 2 (a) Percentage of cuttings that formed adventitious roots and (b) cumulative number of rooted cuttings produced per stock plant after temperature of *Eucalyptus cloeziana* stock plants was changed from 28 to 18, 23 or 33 °C; Avg refers to the average rooting percentage across the five harvests; temperature treatment means (± SE) with different letters are significantly different, *indicates significant decline from the first harvest (ANOVA and LSD test, $p < 0.05$, n = 11–14)

autumn and spring (Catesby & Walker 1998). These percentages were lower than the rooting percentages obtained (17– 100%) using *E. cloeziana* coppice shoots from 5- and 15-year-old felled trees in Brazil (Almeida et al. 2007).

The final numbers of rooted cuttings produced by *E. cloeziana* were clearly related to the number of cuttings produced by stock plants and their rooting percentages. These results are very similar to those of *E. dunnii*, in which stock plant temperature affects shoot production and subsequent adventitious root formation, but different from *C. citriodora*, in which low stock plant temperatures primarily affect shoot production rather than adventitious rooting (Trueman et al. 2013). The results from *E. cloeziana* and *E. dunnii* support the first of the two parts of the

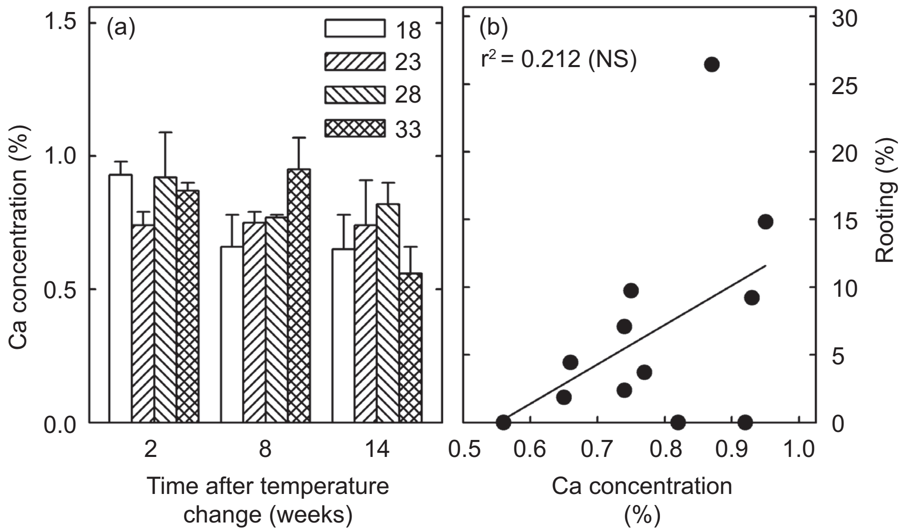

Figure 3 (a) Ca concentration of cuttings and (b) regression between the percentage of cuttings that formed roots and Ca concentration after temperature of *Eucalyptus cloeziana* stock plants was changed from 28 to 18, 23 or 33 °C; Ca concentration (± SE) did not vary significantly between temperature or harvest (ANOVA, $p > 0.05$, $n = 3$); the regression is not significant (NS) ($p > 0.05$, $n = 12$)

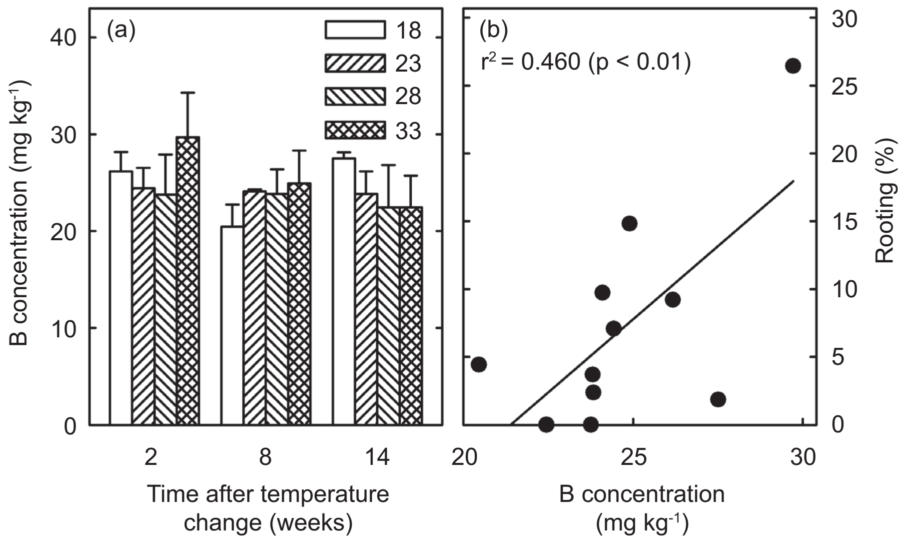

Figure 4 (a) B concentration of cuttings and (b) regression between the percentage of cuttings that formed roots and B concentration, after temperature of *Eucalyptus cloeziana* stock plants was changed from 28 to 18, 23 or 33 °C; B concentration (± SE) did not vary significantly between temperatures or harvests (ANOVA, $p > 0.05$, $n = 3$); the regression is significant ($p < 0.01$, $n = 12$)

hypothesis, described by Assis et al. (2004), that: (1) a decline in production of *Eucalyptus* rooted cuttings at the onset of winter can be attributed to low rooting percentages and (2) low rooting percentages result from a decline in Ca uptake into the shoots of stock plants at low temperatures.

We found, however, that low stock plant temperatures did not reduce the Ca concentration of *E. cloeziana* cuttings and that

Table 1 Correlation coefficients between nutrient concentrations in cuttings of *Eucalyptus cloeziana*

Nutrient	N	P	K	Ca	B	Al	Fe	Mg	Mn	Na	S	Zn
N	–	0.536*	0.790*	0.075	0.383*	0.456*	0.541*	0.306	0.219	-0.352*	0.729*	0.547*
P	0.536*	–	0.448*	0.435*	0.476*	0.337*	0.215	0.254	0.534*	-0.141	0.405*	0.496*
K	0.790*	0.448*	–	0.167	0.578*	0.354*	0.513*	0.471*	0.044	-0.164	0.732*	0.298
Ca	0.075	0.435*	0.167	–	0.342*	-0.120	-0.042	0.333*	0.434*	0.115	0.226	-0.048
B	0.383*	0.476*	0.578*	0.342*	–	0.189	0.450*	0.735*	0.429*	0.122	0.503*	0.258
Al	0.456*	0.337*	0.354*	-0.120	0.189	–	0.322	0.051	0.124	-0.105	0.455*	0.609*
Fe	0.541*	0.215	0.513*	-0.042	0.450*	0.322	–	0.618*	0.264	-0.026	0.657*	0.416*
Mg	0.306	0.254	0.471*	0.333*	0.735*	0.051	0.618*	–	0.311	0.368*	0.600*	0.184
Mn	0.219	0.534*	0.044	0.434*	0.429*	0.124	0.264	0.311	–	-0.066	0.205	0.426*
Na	-0.352*	-0.141	-0.164	0.115	0.122	-0.105	-0.026	0.368*	-0.066	–	-0.024	-0.110
S	0.729*	0.405*	0.732*	0.226	0.503*	0.455*	0.657*	0.600*	0.205	-0.024	–	0.380*
Zn	0.547*	0.496*	0.298	-0.048	0.258	0.609*	0.416*	0.184	0.426*	-0.110	0.380*	–

* Significant (Pearson's correlation, $p < 0.05$, $n = 12$)

the concentrations of other nutrients were also unaffected by the stock plant temperature. The effects of individual nutrients on rooting cannot be clearly delineated because individual nutrient concentrations are often correlated with one another. Nonetheless, rooting percentages were related significantly to the concentrations of just four nutrients, N, P, K and B, with the highest amount of variation in rooting percentage being related to B concentration. The relationship between rooting and B concentration in *E. cloeziana* cuttings strongly reflects those found in *E. dunnii* and *C. citriodora* cuttings, with rooting consistently related to B and Al concentrations in *E. dunnii* and B, P, K and Na concentrations in *C. citriodora* (Trueman et al. 2013). The current results are also similar to those observed in a *E. grandis* × *E. urophylla* clone, in which rooting percentage is related positively to the concentration of B in cuttings but not to the concentrations of N, P, K, Ca, Mg, S, Zn, Fe or Mn (Cunha et al. 2009a).

Few studies have attempted to determine the specific effects of B on adventitious root formation in woody plants (Li et al. 2009, Xavier et al. 2009). Further research is warranted to manipulate B concentrations independently of other nutrients and then assess B effects on adventitious root formation. Boron supply to cuttings can be manipulated through the application of boric acid (H_3BO_3), borax ($Na_2B_4O_710H_2O$) or other sodium borates such as $Na_2B_4O_75H_2O$ (Byers et al. 2001, Schwambach et al. 2005, Valmorbida & Lessa 2008). These compounds are rapidly leached under mist irrigation because of their high solubility. Therefore less-soluble sources of B such as colemanite ($Ca_2B_6O_{11}5H_2O$), ulexite ($NaCaB_5O_98H_2O$) or hydroboracite ($CaMgB_6O_{11}6H_2O$) are often used instead to provide a prolonged release of B (Byers et al. 2001). In practice, though, many commercial nurseries will manipulate nutrient levels by increasing the supply of more-complete fertilisers that contain a broad spectrum of macro- and micronutrients (Santos et al. 2008, Cunha et al. 2009a, c). This strategy may also be effective as B concentrations in the current study are correlated with the concentrations of other nutrients (N, P, K, Ca, Fe, Mg, Mn and S) and rooting percentages are also correlated

Table 2 Coefficients of determination (r^2) for linear regressions between rooting percentage and nutrient concentration in cuttings of *Eucalyptus cloeziana*

Nutrient	r^2
N	0.293*
P	0.255*
K	0.391*
Ca	0.212
B	0.460**
Al	0.035
Fe	0.033
Mg	< 0.001
Mn	0.120
Na	0.028
S	0.149
Zn	0.056

Significant at $p < 0.05$ (*) or $p < 0.01$ (**) (linear regression, n = 12)

with the concentrations of other nutrients (N, P and K).

In conclusion, the most effective stock plant temperature for rooted cutting production of *E. cloeziana* was 33 °C. The effects of lower temperatures on rooting were not the result of reduced Ca or B concentrations in cuttings. We did find underlying positive relationships between adventitious root formation and B concentration. Stock plants of *E. cloeziana* could produce, on average, 12 ± 3 rooted cuttings over a 14-week harvest, sufficient to maintain rooted cuttings in a nursery clonal archive and establish field tests for clonal selection (Oliveira et al. 2006, Trueman & Richardson 2007). However, further research is required to develop propagation techniques for plantation establishment because average rooting percentages (up to 14%) are far below the 70% level expected by commercial nurseries (Trueman 2006, Hunt et al. 2011).

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