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Nutrient partitioning among the roots, hedge and cuttings of *Corymbia citriodora* stock plants

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Abstract

Many eucalypt species are difficult to propagate as rooted cuttings. The mineral nutrition of cuttings is a key factor that limits adventitious root induction but little is known about partitioning of nutrients by eucalypt stock plants. This study determined N, P, K, Ca, B, S, Mg, Mn, Zn, Al, Fe and Na concentrations in the root system, pruned hedge and harvested cuttings of stock plants of the eucalypt, *Corymbia citriodora*. Between 17% and 31% of total plant mass was collected as cuttings at each harvest. The mobile nutrients, N, K and S, were highly concentrated in the cuttings and were removed in high amounts (e.g. 27–46% of total plant N) at each harvest, whereas less-mobile nutrients such as Ca and Zn were less concentrated in the cuttings than other plant parts. Adventitious rooting of eucalypt cuttings has been related to B concentration but this study revealed that B was much more highly concentrated in the hedge than the cuttings. Management of N and K concentrations for shoot production, and B concentrations for adventitious rooting, may be critical for sustaining rooted cutting production by *C. citriodora*.

Keywords: Boron, cloning, *Eucalyptus*, mineral nutrition, Myrtaceae, propagation

1. Introduction

The cuttings of some eucalypt species from riparian or high-rainfall habitats have a high capacity for forming adventitious roots (Wendling and Xavier, 2005; Saya *et al*., 2008; Goulart *et al.*, 2011) but most plantation eucalypts are considered difficult to propagate from cuttings (Assis *et al*., 2004; Brondani *et al.*, 2011, 2012a; Kilkenny *et al*., 2012). Difficulties with clonal propagation of woody plants have often been overcome by developing improved methods for managing their nursery stock plants and treating their harvested cuttings (Leakey, 2004 ; Pohio *et al*., 2005; Wendling *et al*., 2010; Majada *et al*., 2011). Mineral nutrition of cuttings is one of the key factors that limit adventitious rooting (Blazich, 1988; Xavier *et al*., 2009). However, in the absence of foliar fertilization, the nutritional status of cuttings is primarily determined by the nutritional status of the stock plant because there may be little nutrient uptake through the cut stump of cuttings before roots are formed (Grange and Loach, 1983; Blazich, 1988; Santos *et al*., 2009).

One hypothesis for low adventitious rooting in subtropical eucalypts has been that Ca uptake into the young shoots of stock plants is limited when temperatures are suboptimal, and that root formation is correlated with Ca concentration (Assis *et al*., 2004). Subsequent studies have found that lowering the stock plant temperature does not affect the Ca concentration, and that rooting is usually not correlated with Ca concentration,in cuttings of three subtropical eucalypt species, *Corymbia citriodora, Eucalyptus cloeziana* and *E. dunnii* (Trueman *et al*., 2013a, b). However, adventitious root induction is consistently correlated with B concentration in cuttings of all three of these species (Trueman *et al*., 2013a, b) and it is often correlated with B concentration in clones of *E. grandis* × *E.urophylla* (Cunha *et al*., 2009a, b). Rooting is also correlated with N, P and K concentrations in *E. cloeziana*, and with P and K concentrations in *C. citriodora*, but it is not correlated with concentrations of these macronutrients in *E. dunnii*, *E. grandis*, *E. grandis* × *E.urophylla* or *E. urophylla* (Cunha *et al*., 2009a, b; Trueman *et al*., 2013a, b). The partitioning of nutrients among the roots, pruned hedge and cuttings of stock plants has been described recently for *E. urophylla* (Neto *et al*., 2012) but, otherwise, little is known about the uptake and distribution of nutrients by eucalypt stock plants, despite their importance for determining the nutrient status and adventitious rooting potential of cuttings.

This study determined the distribution of N, P, K, Ca, B, S, Mg, Mn, Zn, Al, Fe and Na among the roots, the pruned hedge and the cuttings of *C. citriodora stock* plants. The aim was to reveal mobile nutrients that are lost from the stock plant at high rates during each harvest of cuttings and less-mobile nutrients that might not be transported to the young shoots in sufficient quantities to ensure high adventitious rooting. The study species is grown widely for its timber, pulp and essential oils (Dillon *et al*., 2012; Dickinson *et al*., 2013; Gbenou *et al*., 2013) but it is considered difficult to propagate as rooted cuttings (Shepherd *et al*., 2007; Trueman and Richardson, 2008; Trueman *et al*., 2013a). The results of this study will assist in developing fertilization strategies for sustainable shoot production and rooted cutting production by stock plants of *C. citriodora*.

2. Materials and Methods

The stock plants harvested in this study were a subsample of those used in the adventitious rooting study of Trueman *et al*. (2013a). Briefly, stock plants of *C. citriodora* subsp. *variegata* were raised in a glasshouse in Gympie (26°11'S, 152°40'E) by sowing seeds in January 2009 in potting mix consisting of a 75/25 (v/v) mixture of shredded pine bark and perlite, with 3kg of 8-9 month slow release Osmocote ™ fertiliser (Scotts International, Heerlen, The Netherlands), 3kg of lime (Unimin, Lilydale, Australia), 1kg of gypsum (Queensland Organics, Narangba, Australia), 1kg of Micromax ™ granular micronutrients and 1kg of Hydroflo ™ soil wetting agent (both from Scotts Australia, Baulkham Hills, Australia) incorporated per m^3. The N, P and K contents of the Osmocote fertiliser were 16.0%, 1.3% and 9.1% (w/w), respectively, and the pH of the potting mix was approximately 6.5.The seedlings were transplanted in February 2009 into 2.8L pots filled with the same potting mix, and then transferred randomly into four controlled-temperature glasshouse chambers in Nambour (26°38'S, 152°56'E). Temperatures in all chambers were set at 28°C/23 °C (day/night; 0600-1800 h/1800-0600 h, respectively). Irradiance was reported by Trueman *et al*. (2013a).

The seedlings were managed as hedged stock plants, commencing in April 2009, by pruning (i.e. hedging) at 3-week intervals to a height of ~30 cm and a canopy diameter of ~20 cm. The temperatures in three of the chambers were changed randomly on 15 June 2009 to provide four temperatures across the four chambers:18 °C/13 °C, 23 °C/18 °C, 28 °C/23 °C and 33 °C/28 °C (day/night). This date was regarded as the commencement of the experiment, consistent with the terminology in Trueman *et al*. (2013a, b).

Temperatures and their corresponding stock plants were relocated randomly to different chambers every 4 weeks to minimise the effects of chamber.

Cuttings were harvested from all stock plants at 2, 5, 8, 11 and 14 weeks after commencement of the experiment (Trueman *et al*., 2013a). For the current study, three stock plants from each temperature(18 °C/13 °C, 23 °C/18 °C, 28 °C/23 °C and 33°C/28 °C) were destructively sampled at 2, 8 and 14 weeks (i.e. 12 stock plants on each sample date). All available cuttings were collected directly from each stock plant, and each stock plant was dissected into five complete parts: roots (R), hedge stems (HS), hedge leaves (HL), cutting stems (CS) and cutting leaves (CL). The roots were rinsed gently in water to remove adhering potting mix. The five plant parts were placed in separate paper bags, dried for 7 d at 65 °C, weighed, and ground using a Retsch MM200 tissue homogeniser (Retsch, Haan, Germany). The concentrations of N and S in each sample were determined by combustion analysis (McGeehan and Naylor, 1988; Rayment and Higginson, 1992) using a LECO CNS 2000. The concentrations of P, K, Al, B, Ca, Fe, Mg, Mn, Na and Zn were determined by inductively coupled plasma – atomic emission spectroscopy (Munter and Grande, 1981) after nitric and perchloric acid digestion (Martinie and Schilt, 1976).

Dry mass, nutrient concentrations and nutrient contents were analysed by 2-way ANOVA, comparing the five plant parts and four temperatures within each harvest date. Two-way ANOVA was used because extensive interactions between harvest date and the other factors (plant part and chamber temperature) were detected by 3-way ANOVA but temperature effects and interactions between plant part and temperature were generally not significant in the 2-way ANOVAs. Post-hoc least significant difference (LSD) tests were performed only when significant differences were detected by ANOVA. Data were square root or log transformed when variance was heterogeneous. Means are reported with standard errors, and mean differences or interactions were regarded as significant at $p < 0.05$.

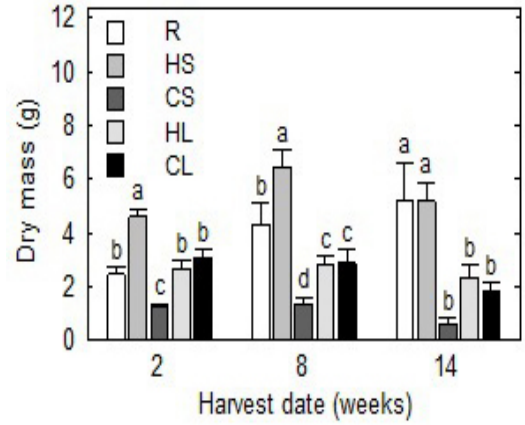

Figure 1. Dry mass partitioning among the roots (R), hedge stems (HS), cutting stems (CS), hedge leaves (HL) and cutting leaves (CL) of *Corymbia citriodora* stock plants at three harvest dates (2, 8 and 14 weeks after commencement of the experiment). Means (+ s.e.) with different letters within a harvest date are significantly different (ANOVA and LSD test, $p < 0.05$, n = 12)

3. Results and Discussion

Much of the stem mass in *C. citriodora* stock plants was located in the main frame work of the hedge (HS) rather than in the cuttings (CS), whereas leaf mass was distributed almost equally between the hedge (HL) and the cuttings (CL) (Figure 1). Root mass (R) increased during the experiment but the mass of available cuttings (CS + CL) declined at the final harvest date, as shown previously from the more-extensive harvests of cuttings from 18–24 stock plants (Trueman *et al*., 2013a). Between 17% and 31% of total plant mass was collected as cuttings at each harvest, with this percentage declining during the experiment (Table 1). These percentages were similar to those from the cuttings of *E. urophylla* stock plants (~18–25%) across a range of N application rates (Neto *et al*., 2012).

Table 1. Biomass and nutrient losses (as percentages of whole-plant biomass or nutrient content) due to the harvest of cuttings from *Corymbia citriodora* stock plants at three sample times after commencement of the experiment.

	Sample time		
	2 weeks	8 weeks	14 weeks
Biomass	31 ± 2	22 ± 3	17 ± 2
N	46 ± 3	30 ± 4	27 ± 3
P	36 ± 3	24 ± 3	17 ± 2
K	42 ± 3	32 ± 4	26 ± 3
Ca	20 ± 3	12 ± 3	8 ± 1
B	31 ± 3	23 ± 4	15 ± 2
S	44 ± 3	29 ± 4	22 ± 2
Mg	36 ± 3	24 ± 3	19 ± 2
Mn	31 ± 3	21 ± 3	13 ± 2
Zn	21 ± 3	15 ± 3	12 ± 2
Al	10 ± 2	6 ± 1	5 ± 1
Fe	21 ± 3	9 ± 1	6 ± 1
Na	5 ± 1	1 ± 0	1 ± 0

Means are provided with SE (n = 12)

Nitrogen was most concentrated in the hedge leaves and cutting leaves (HL and CL) and least concentrated in the stems of the hedge (HS) (Figure 2a), so that between 32 ± 4 and 88 ± 7 mg of N (CS + CL) was lost from the stock plant during each harvest of cuttings (Figure 2b). Phosphorus concentration varied little within the plant (Figure 2c) and so the allocation of P (Figure 2d) reflected the allocation of dry mass to different parts of the stock plant (Figure 1; Table 1). Potassium was most concentrated in the leaves (HL and CL) and the cutting stems (CS) (Figure 2e), and stock plants lost between 33 ± 5 and 78 ± 9 mg of K (CS + CL) at each harvest (Figure 2f). Therefore, disproportionately high amounts of total plant N (27–46%) and K (26–42%) were removed at each harvest (Table 1). These nutrients are highly mobile and are often concentrated in growing tissues such as young shoots (Karley and White, 2009; Li *et al*., 2010). Much greater percentages of total plant N (~75–86%) are removed during the harvest of *E. urophylla* cuttings, which also lose disproportionately high amounts of total plant P (~33–56%) and K (~30–43%) at each harvest (Neto *et al*., 2012). Replacement of these mobile macronutrients may be critical for sustained stock plant growth as well as for root formation by cuttings, since adventitious rooting has been correlated with P and K concentrations in the cuttings of *C. citriodora* (Trueman *et al*., 2013a) and with N, P and K concentrations in *E. cloeziana* (Trueman *et al*., 2013b). However, rooting percentages are not correlated with N, P and K concentrations in the cuttings of *E. dunnii, E. grandis, E. grandis* × *E.urophylla* and *E. urophylla* (Cunha *et al.*, 2009 a, b; Trueman *et al*., 2013a).

Calcium concentrations were much higher in the roots (R) and the hedge (HS and HL) than in the cuttings (CS and CL) (Figure 3a). Therefore, Ca loss through the harvest of cuttings (Figure 3b) was lower as a proportion of the plant's total nutrient content than it was for N, P and K (Figures 2b, 2d, 2f; Table 1). In fact, disproportionately low amounts of Ca (8–20%) were lost through the harvest of cuttings (Table 1) in contrast with high losses (~24–43%) observed with *E. urophylla* (Neto *et al*., 2012). Ca is considered an immobile nutrient that tends to be retained at high levels in mature and senescing organs (McLaughlin and Wimmer, 1999; Karley and White, 2009). Its relatively low concentration in *C. citriodora* cuttings (CL: 0.73± 0.07% to 0.79± 0.07%; Figure 3a) could be a cause of reported low rooting percentages in this species (Shepherd *et al.*, 2007; Trueman and Richardson, 2008; Trueman *et al*., 2013a). No correlation has been found between Ca concentration and rooting percentage in *C. citriodora* across a concentration range in whole cuttings of 0.57% to 1.12% (Trueman *et al*., 2013a) or in studies with *E. cloeziana, E. dunnii, E. grandis* × *E.urophylla* and *E. urophylla* (Cunha *et al*., 2009 a, b) Trueman *et al*., 2013a, b).

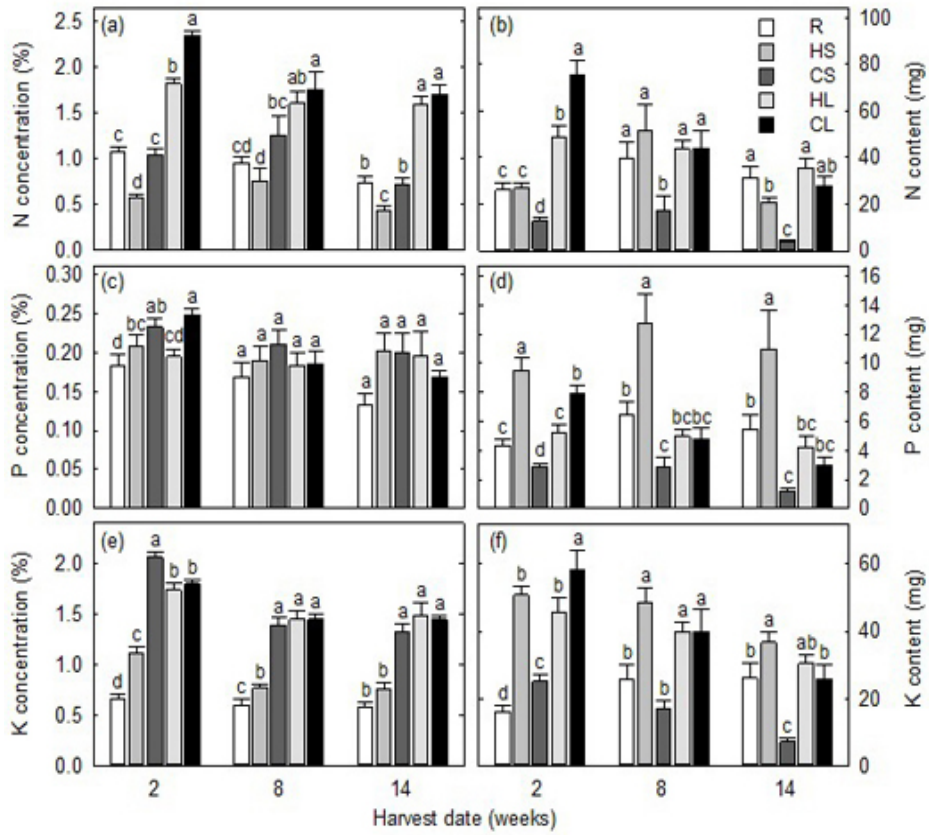

Figure 2. N, P and K partitioning among the roots (R), hedge stems (HS), cutting stems (CS), hedge leaves (HL) and cutting leaves (CL) of *Corymbia citriodora* stock plants at three harvest dates (2, 8 and 14 weeks after commencement of the experiment). Means (+ s.e.) with different letters within a harvest date are significantly different (ANOVA and LSD test, $p < 0.05$, n = 12)

However, the Ca concentrations in *C. citriodora* cuttings in these studies were much lower than concentrations in the current hedge leaves (1.90 ± 0.11% to 2.00 ± 0.16%; Figure 3a), and so there may be scope to improve adventitious rooting if Ca levels in the upper shoots can be increased greatly using foliar fertilizers, Ca supplements such as lime or gypsum, or optimised climatic regimes that increase Ca mobility.

Boron was more highly concentrated in the hedge leaves (HL) than the cutting leaves (CL) or other plant parts (R, HS and CS) (Figure 3c), resulting in much of the B content of the stock plant being confined to the hedge (HL and HS; Figure 3d). The proportion of total plant B removed through the harvest of cuttings (15–31%) reflected the biomass allocation to cuttings (Table 1), as it did during the harvest of cuttings from *E. urophylla* (Neto *et al*., 2012). B is an immobile nutrient in many plant species where sucrose is the primary photoassimilate, but it is mobile in species that contain polyols such as sorbitol and mannitol (Miwa *et al.*, 2009; Lehto *et al*., 2010)

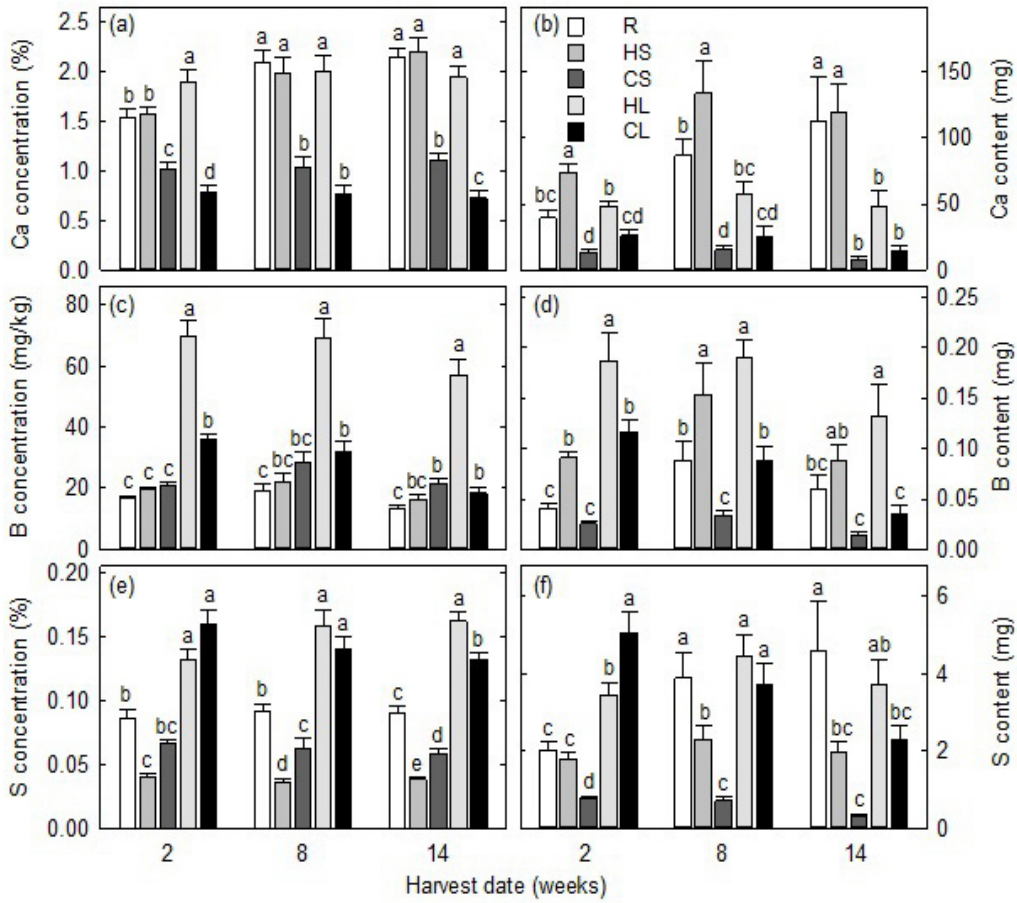

Figure 3. Ca, B and S partitioning among the roots (R), hedge stems (HS), cutting stems (CS), hedge leaves (HL) and cutting leaves (CL) of *Corymbia citriodora* stock plants at three harvest dates (2, 8 and 14 weeks after commencement of the experiment). Means (+ s.e.) with different letters within a harvest date are significantly different (ANOVA and LSD test, $p < 0.05$, n = 12)

E. grandis and E. grandis × *E. urophylla* are rich in sorbitol and mannitol (Leite *et al*., 2008, 2010), and B appears to be mobile in *E. grandis* × *E. urophylla* (Mattiello *et al.,* 2009). Consistent relationships between B concentration and rooting percentage have been found in cuttings of *C. citriodora, E. cloeziana* and *E. dunnii (*Trueman *et al.,* 2013a, b*),* and rooting percentages in three *E. grandis* × *E. urophylla* clones have been related to the concentration of B but not N, P, K or Ca (Cunha *et al*.,2009a, b). The effect of manipulating B concentration independently of other nutrients has rarely been assessed for adventitious root formation (Josten and Kutschera, 1999; Li *et al*., 2009; Xavier *et al.*, 2009) but there appears to be great potential to improve rooted cutting production by increasing B levels in the young leaves of stock plants

(i.e. in the cuttings). Boron can be supplied to stock plants as boric acid (H_3BO_3), borax ($Na_2B_4O_7 \cdot 10H_2O$), other sodium borates such as $Na_2B_4O_7 \cdot 5H_2O$, or through less-soluble sources such as colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$), ulexite ($NaCaB_5O_9 \cdot 8H_2O$) or hydroboracite ($CaMgB_6O_{11} \cdot 6H_2O$) that provide a more-prolonged release (Byers *et al.*, 2001; Fageria *et al.*, 2009). Supplementation with boric acid increases stock plant survival, shoot production and shoot quality in *E. benthamii* (Brondani *et al.*, 2012b).

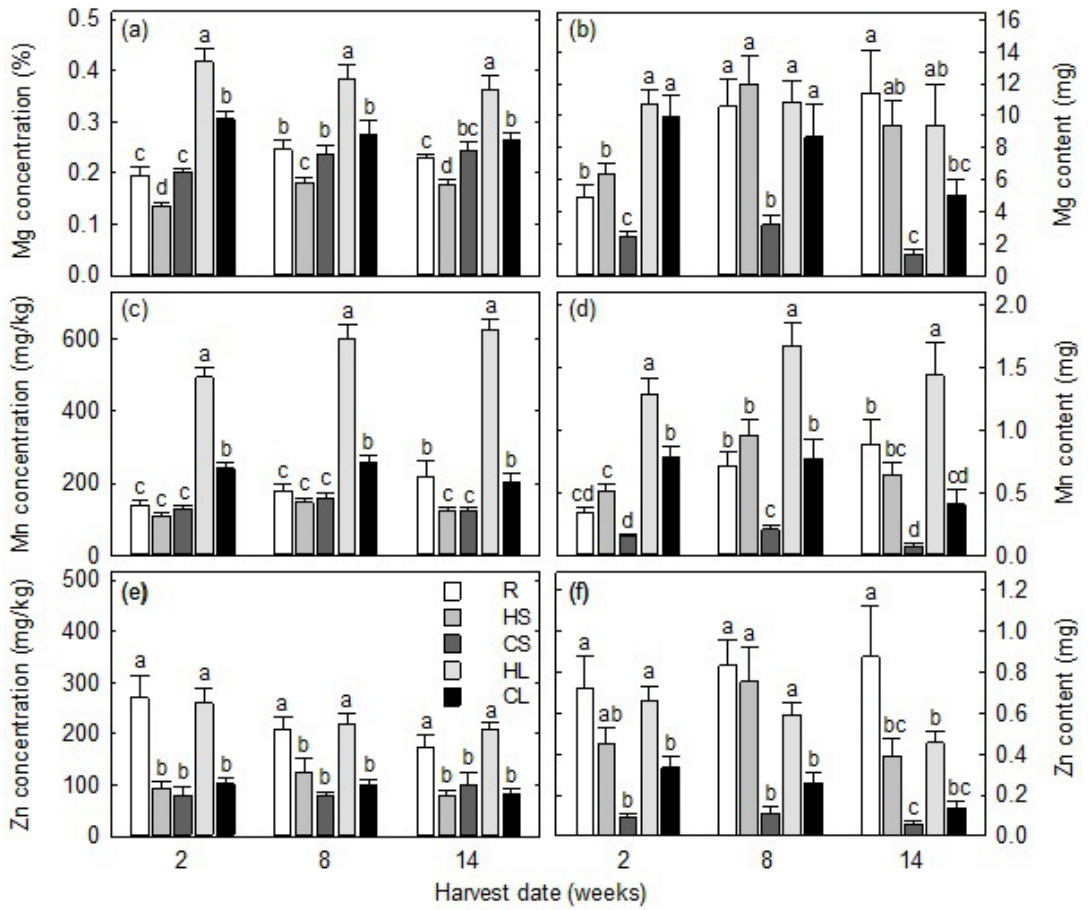

Figure 4. Mg, Mn and Zn partitioning among the roots (R), hedge stems (HS), cutting stems (CS), hedge leaves (HL) and cutting leaves (CL) of *Corymbia citriodora* stock plants at three harvest dates (2, 8 and 14 weeks after commencement of the experiment). Means (+ s.e.) with different letters within a harvest date are significantly different (ANOVA and LSD test, $p < 0.05$, n = 12)

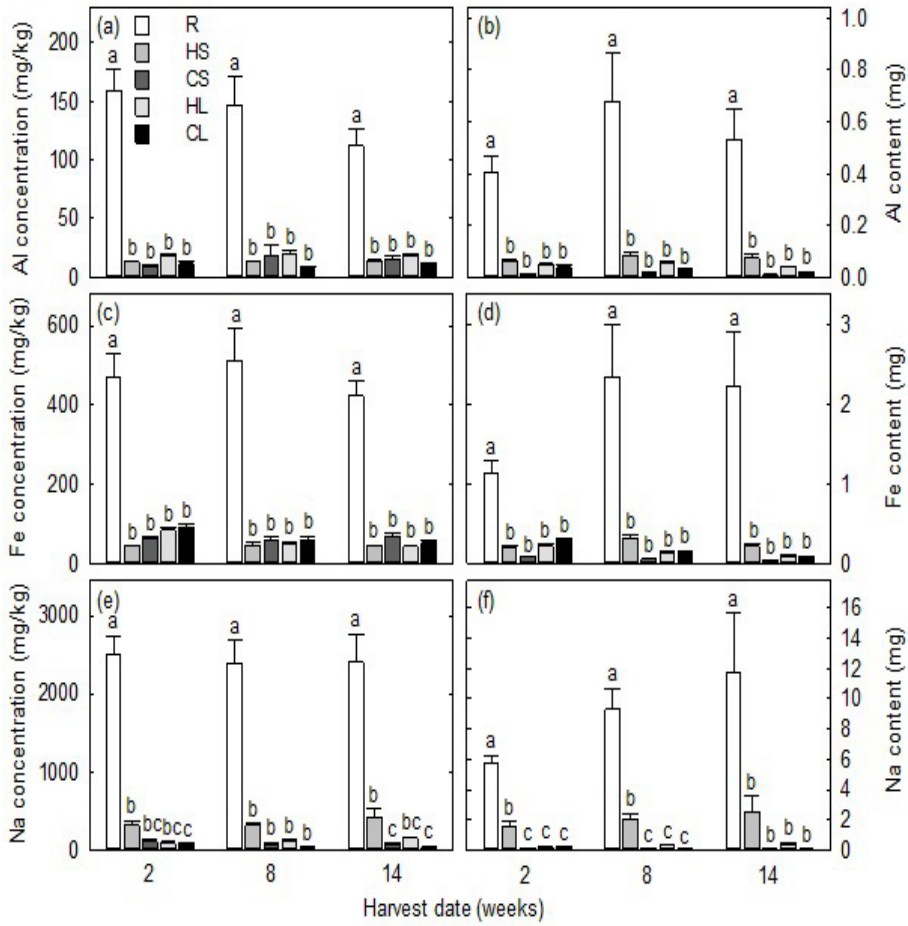

Figure 5. Al, Fe and Na partitioning among the roots (R), hedge stems (HS), cutting stems (CS), hedge leaves (HL) and cutting leaves (CL) of *Corymbia citriodora* stock plants at three harvest dates (2, 8 and 14 weeks after commencement of the experiment). Means (+ s.e.) with different letters within a harvest date are significantly different (ANOVA and LSD test, $p < 0.05$, n = 12)

Sulphur was also concentrated most highly in the leaves (Figures 3e, 3f), but with little or no difference in S concentration between the hedge leaves (HL) and cutting leaves (CL) (Figures 3e). Magnesium and manganese were concentrated most highly in the hedge leaves (HL) and, on some occasions, the cutting leaves (CL) (Figures 4a–d).

Zinc was concentrated most highly in the hedge leaves (HL) and the roots (R) (Figures 4e, 4f). Disproportionately high amounts of total plant S (22–44%) were removed from the stock plant as cuttings (Table 1), but S removal was not as high as the amount harvested (~74–84% of total plant S) in the cuttings of *E. urophylla* (Neto *et al*., 2012). Removals of Mg and

Mn in cuttings were in proportion to the removal of biomass, while low proportions of total plant Zn (12–21%) were in the cuttings (Table 1), similar to the low proportions (~8–12%) found in *E. urophylla* cuttings (Neto *et al*., 2012). S and Mg are mobile nutrients that are often highly concentrated in young leaves (Hawkesford and De Kok, 2006; Karley and White, 2009; Maathuis, 2009). Mn is immobile in many species andis often highly concentrated in older leaves (Dučic *et al*., 2006; Page *et al*., 2006, 2012) including those of eucalypts, which are often considered as Mn accumulators (Hill *et al.*, 2001; Jobbágy and Jackson, 2004). Zn mobility is considered to be intermediate between highly mobile elements such as K and P and immobile elements such as Ca, but Zn is often highly concentrated in roots and older leaves (Page and Feller, 2005; Ivanov *et al*., 2011). Rooting percentages have been positively correlated with Zn concentrations in the cuttings of three *E. grandis* × *E. urophylla* clones (Cunha *et al*., 2009b) but not in *C. citriodora, E. cloeziana*, *E. dunnii*, *E. grandis* or *E. urophylla* (Cunha *et al*., 2009 a, b; Trueman *et al*., 2013a, b). However, the Zn concentrations in *E. grandis* × *E. urophylla* cuttings (36–45 mg/kg) were much lower than those in *C. citriodora* (Figure 4e).

Aluminium, iron and sodium were concentrated more highly in the roots (R) than in any of the above-ground plant parts, so that most of the Al, Fe and Na content of the plant was contained within the roots and only a low proportion was within the cuttings (Figure 5). The proportion (6–21%) of total plant Fe in the cuttings (Table 1) was similar to the low proportion (9–15%) in *E. urophylla* cuttings (Neto *et al*., 2012). Fe is an actively-transported nutrient that is required for photosynthesis and mitochondrial respiration (Hänsch and Mendel, 2009; Puig and Peñarrubia, 2009) but is often concentrated most highly in the roots (Gomes *et al*., 2012; Neto *et al*., 2012). Al is also concentrated in roots and returned to the soil through a variety of efflux mechanisms, but some plants immobilize Al in the root symplast external to the endodermis (Vázquez, 2002; Nguyen *et al*., 2005; Zheng *et al.*, 2005). Na is also concentrated in the roots and excluded from reaching the shoots of many species to maintain high photosynthetic rates (Hauser and Horie, 2010; Edelstein *et al*., 2011). Rooting percentages are generally not correlated with Fe concentration in eucalypt cuttings (Cunha *et al*., 2009 a, b; Trueman *et al*., 2013a, b), but rooting has been correlated positively with Na and Al concentrations in the cuttings of *C. citriodora* and *E. dunnii,* respectively (Trueman *et al*., 2013a).

4. Conclusion

This study has revealed the partitioning of biomass and nutrients among the roots, hedge and cuttings of Corymbia stock plants. Between 17% and 31% of total plant mass was harvested from the stock plants at each collection of cuttings, but some mobile nutrients (N, K and S) were highly concentrated in the cuttings and were disproportionately lost from the stock plant during each harvest of cuttings. More-immobile elements (Ca and Zn) were less concentrated in the cuttings than other parts of the stock plant. Importantly, B was more highly concentrated in leaves of the hedge than in leaves of the cuttings. Short-term management of B concentration in cuttings and longer-term management of the N and K supply to stock plants may be critical for sustaining high levels of rooted cutting production by *C. citriodora* and other eucalypt species.

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