Relationships between Indole-3-Butyric Acid, Photoinhibition and Adventitious Rooting of *Corymbia torelliana*, *C. citriodora* and F₁ Hybrid Cuttings

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ABSTRACT

Rooted cuttings propagation is widely used for maximising tree yield, quality and uniformity in conjunction with clonal selection. Some eucalypt species are deployed as rooted cuttings but many eucalypts, such as the spotted gums (e.g. *Corymbia citriodora*), are considered too difficult-to-root to be produced as cuttings. This study examined IBA effects on photoinhibition, root formation, mortality, and root and shoot development of cuttings of *C. torelliana*, *C. citriodora* and their widely-planted F₁ hybrids. IBA had little or no effect on photoinhibition but it had strong, dose-dependent effects on root formation and mortality. IBA frequently increased primary root number of rooted cuttings but it did not increase total root weight, length, surface area or volume, possibly because the highest dose (8 g IBA/kg powder) caused leaf abscission and sometimes reduced leaf area (by 55–79%) or shoot dry weight (by 40–58%). An intermediate dose (3 g IBA/kg powder) most consistently improved root formation with little or no effect on mortality or shoot development. Across the F₁ hybrid families, this treatment increased the number of rooted cuttings by 72–121% and more than doubled the number of primary roots per rooted cutting (from 1.1–1.7 roots to 3.5–4.1 roots). This simple treatment will facilitate commercial multiplication of superior individuals or selected families of *C. torelliana* × *C. citriodora* through a vegetative propagation system.

INTRODUCTION

Clonal selection and rooted cuttings propagation are widely used for maximising growth, quality and uniformity in tree production systems. Rooted cuttings propagation is also used when seed supply is limited by poor or irregular flowering (Leakey *et al.* 1994) or where seed production is constrained by the need for hand-pollination, such as in the production of hybrids. Among candidates for large-scale clonal propagation are the eucalypts, *Eucalyptus*, *Corymbia* and *Angophora*, which are the world's most widely planted hardwood trees because of their large number of species, wide adaptability to soils and climate, and fast growth rates (Teulières *et al.* 2007). Some eucalypts, such as *E. deglupta*, *E. grandis* and *E. saligna*, are highly amenable to vegetative propagation (Eldridge *et al.* 1994; Fogaça and Fett-Neto 2005; Wendling and Xavier 2005). Many other eucalypts are difficult to propagate as rooted cuttings (Eldridge *et al.* 1994; Luckman and Menary 2002; Assis *et al.* 2004; Fogaça and Fett-Neto 2005; Schwambach *et al.* 2008).

The spotted gums, *Corymbia citriodora*, *C. henryi* and *C. maculata*, are an important series of plantation eucalypts that are considered difficult to propagate vegetatively (Shepherd *et al.* 2007; Barbour *et al.* 2008; Shepherd *et al.* 2008). Hardwood plantation establishment in subtropical eastern Australia is based heavily on spotted gum seedlings (Lee 2007; Barbour *et al.* 2008). Rapid plantation expansion, coupled with sparse and irregular flowering in seed orchards and natural stands of desired provenances, has constrained seed supply of spotted gums and limited plantation development (Lee 2007). Plantations are also established using inter-series hybrids that combine disease tolerance from *C. torelliana* with the excellent wood quality and stem

form of *C. citriodora* (Lee 2007). Eucalypt hybrids are widely grown because they often exhibit heterosis, perform well outside the natural range of their parent species, and combine desirable characteristics of their parents (Potts and Dungey 2004; Shepherd *et al.* 2007). *Corymbia* hybrids are produced by hand-pollination, and demand for hybrid germplasm is much greater than current seed supply. Large-scale commercial deployment of *Corymbia* hybrids requires an efficient system for vegetative propagation to allow multiplication of superior individuals or selected families as rooted cuttings (Lee 2007; Shepherd *et al.* 2007).

Application of auxin, in particular indole-3-butyric acid (IBA), is the most common and possibly most effective treatment to enhance rooting of stem cuttings (Blazich 1988a; Hartmann *et al.* 1997; Leakey 2004). IBA has been used to increase the percentage of cuttings that form roots, or the number of roots formed per cutting, in a wide range of tree species (Henrique *et al.* 2006; Husen and Pal 2007; Husen 2008) including eucalypts (Wendling *et al.* 2000; Zuffellato-Ribas and Rodrigues 2001; Luckman and Menary 2002; Corrêa *et al.* 2005; Fogaça and Fett-Neto 2005). However, cuttings of many species or clones appear unresponsive to auxin (Shiembo *et al.* 1996; Wendling and Xavier 2005; Atangana *et al.* 2006; Trueman and Peters 2006) and supra-optimal doses can cause cutting death (Ofori *et al.* 1996; Perry and Trueman 1999; Zuffellato-Ribas and Rodrigues 2001; Wendling and Xavier 2005).

Cuttings must survive physiological stress after severance from the stock plant, with little uptake of water and nutrients until roots penetrate the propagation medium (Grange and Loach 1983; Blazich 1988b; Hartmann *et al.* 1997). Under these conditions, leaves of unrooted cuttings can experience photoinhibition (Mesén *et al.* 1997, 2001;

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Bruce *et al.* 2001; Pohio *et al.* 2005); i.e. the reduction in quantum efficiency of photosynthesis that occurs when leaves absorb light energy in excess of the capacity of the photosynthetic apparatus (Osmond and Grace 1995).

Photoinhibition initially involves regulated dissipation of excess excitation energy, but can also result from damage or inactivation of photosystem II reaction centres (Adams and Demmig-Adams 1994; Osmond and Grace 1995; Skillman and Osmond 1998). The maximum photochemical efficiency of photosystem II (F_V/F_M) under optimal conditions is approximately 0.83 for most species, with lower values indicating exposure to stress (Björkman and Demmig 1987; Maxwell and Johnson 2000). Low mean F_V/F_M values (0.12–0.60 and 0.45–0.59) have been reported for cuttings of two tropical tree species, in which low rooting percentages were related to low F_V/F_M (Mesén *et al.* 1997, 2001). Little or nothing is known about the effects of applied auxin on photoinhibition of cuttings despite the widespread use of exogenous auxin in clonal propagation systems and the potential for auxin to induce additional physiological stress.

The objective of this study was to assess IBA requirements for rooting of *C. torelliana*, *C. citriodora* and hybrid cuttings, and specifically whether (1) IBA caused photoinhibition; (2) IBA affected the percentage of cuttings that formed roots; (3) high IBA doses caused cutting mortality; and (4) IBA altered root or shoot development of rooted cuttings. These results would assist in developing a vegetative propagation system for industrial deployment of *C. torelliana* × *C. citriodora* rooted cuttings.

MATERIALS AND METHODS

Stock plants

Seeds of *C. torelliana*, *C. citriodora* subsp. *variegata*, and three F$_1$ full-sibling hybrid families were obtained from the Hardwood Tree Improvement Group, Department of Primary Industries and Fishe-

ries, Queensland. The hybrid families were 1CT2-013 × 1CV2-114 ('13'), 1CT2-016 × 1CV2-105 ('16') and 1CT2-030 × 1CV2-054 ('30'). Two of these families, 13 and 16, were also used for the *in vitro* propagation study of Trueman and Richardson (2007). *C. torelliana* seeds were from an open-pollinated bulk seed lot of the same three mother trees, 1CT-013, 1CT-016 and 1CT-030, in approximately equal proportions. *C. citriodora* seeds were from an open-pollinated bulk seed lot of 19 trees of the same provenance (Woondum) as the three father trees.

Seeds were sown in potting mix with a thin covering of vermiculite in July 2005, and germinated under mist irrigation in a glasshouse in Gympie (26°11'S, 152°40'E), Queensland. In August 2005, at least 160 seedlings of each species and hybrid family (i.e. five taxa) were gently removed from the potting mix, washed, placed into 90-cm^3 tubes containing perlite, and transferred to a translucent white polyethylene chamber. Irradiance and temperature in the glasshouse and polyethylene chamber were monitored between May and October 2006 (**Fig. 1**). The chamber was equipped with an intermittent flooding system based on the hydroponics mini-hedge and mini-cuttings systems developed for *Eucalyptus* clonal propagation in Brazil (Wendling *et al.* 2003; Assis *et al.* 2004; Schwambach *et al.* 2008). Tubes were inundated with nutrient solution for 5 min every 2 hours from 0600 H to 1200 H, every hour from 1200 H to 1600 H, and every 3 hours from 1800 H to 0600 H. The nutrient solution comprised 4.03 mM Ca(OH)$_2$, 2.01 mM K$_2$SO$_4$, 0.76 mM MgSO$_4$, 0.55 mM KH$_2$PO$_4$, 0.15 mM NH$_4$NO$_3$, 0.11 mM Fe·EDTA, 41.24 µM H$_3$BO$_3$, 33.15 µM Mn·EDTA, 6.05 µM Zn·EDTA, 0.72 µM Cu·EDTA and 0.41 µM Na$_2$MoO$_4$. Fresh solutions were provided every 2 weeks, with initial pH adjusted to 6.0 using HCl or NaOH. Seedlings were maintained as mini-hedges between 5-cm and 20-cm height by regular pruning.

Experimental design

Cuttings, comprising the distal 5-cm of vertically-oriented branches, were collected from the mini-hedges on two occasions: 31 May – 5 Jun 2006 (winter experiment) and 18 Sep – 21 Sep 2006
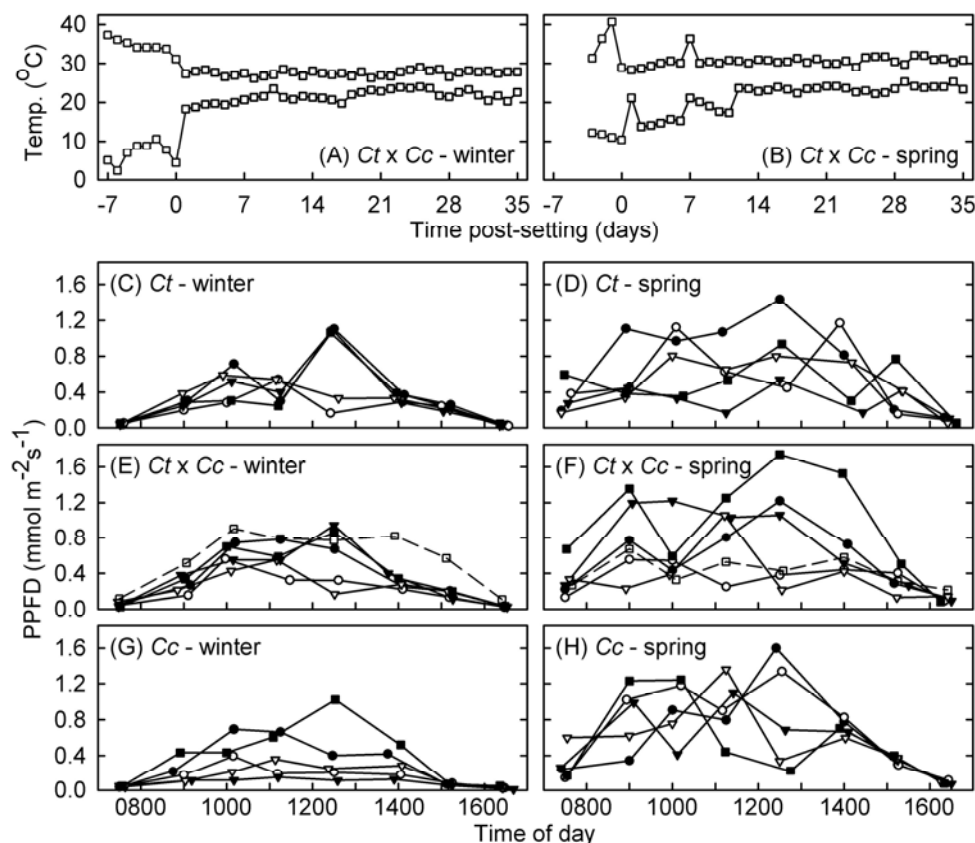

Fig. 1 (A, B) Daily maximum and minimum temperatures in the greenhouse (pre-setting) and glasshouse (post-setting), and **(C-H)** photosynthetic photon flux densities (PPFD) in the greenhouse (□ 1 d pre-setting; dashed lines) and glasshouse (● 1, ○ 8, ▼ 15, ▽ 25 and ■ 34 d post-setting; solid lines) for *C. torelliana* (*Ct*), hybrid family 13 (*Ct* × *Cc*) and *C. citriodora* (*Cc*) cuttings set in winter or spring.

(spring experiment). Commercial collection of *Corymbia* cuttings occurs throughout the year in subtropical Queensland. Cuttings from all mini-hedges within each taxon were collected, mixed randomly, and set on the same day but setting of the five taxa occurred across several days. The number of cuttings available for each taxon ranged from 250 to 370 per experiment. Ten cuttings per taxon in each experiment were selected randomly for measurement of their leaf area (pre- and post-pruning) and dry weight (post-pruning). Pruning involved removal of half to two-thirds of the length of each expanded leaf. All leaf portions were retained, scanned, and their areas analysed (without subtending petiole or stem tissue) using a WinRHIZO LA1600+ scanner with WinFOLIA PRO v.2004a image analysis software (Régent Instruments Inc, Ste-Foy, Canada). The stem, petiole and unpruned leaf portions of each cutting were then placed together in a paper bag, dried for several days at 65°C, and weighed.

The remaining cuttings of each taxon (240-360 per experiment) were pruned as above and allocated randomly to one of four hormone treatments: 0, 1, 3 or 8 g IBA/kg talcum powder. Cuttings were treated and set in replicate rows of five cuttings, with the 12-18 replicate rows of each treatment distributed randomly among the propagation trays. Cuttings were dipped 0.5-cm into treatment powder for about 1 s and placed 1-cm deep in a 90-cm^3 tube containing a 75/25 (v/v) mix of perlite and shredded pine bark with 3 kg of 8-9 month slow-release Osmocote^{TM} fertiliser (Scotts International, Heerlen, The Netherlands) and 1 kg of gypsum incorporated per m^3. Trays were placed under mist irrigation in an adjacent glasshouse, with mist provided for 10 s every 15 min during the day (0600 H to 1800 H) and 10 s every 20 min at night (1800 H to 0600 H). Temperatures were recorded for the duration of experiments using Tinytalk dataloggers (RS Components, Smithfield, Australia) (**Fig. 1A, 1B**). Irradiance was determined using a quantum sensor (Delta-T Devices Ltd, Cambridge, UK) on the days when chlorophyll fluorescence of cuttings was recorded (**Fig. 1C-H**).

Chlorophyll fluorescence

Chlorophyll fluorescence (F_V/F_M) was measured for three of the taxa: *C. torelliana*, *C. citriodora* and hybrid family 13. Fluorescence was recorded at 0800 H and 1300 H from adaxial leaf surfaces of 20 shoots per taxon on a single day prior to setting and of 60 cuttings per taxon at 1 d, 8 d, 15 d, 25 d and 34 d post-setting. The 60 cuttings were selected on each occasion to include 15 cuttings from each of the four hormone treatments. Dead or defoliated cuttings were not included. Leaves were dark adapted for 15 min, and fluorescence values were obtained with a 5-s flash of 100% of the available light (approx. 3 mmol photons m^{-2} s^{-1}) using a Fluorescence Induction Monitor (Analytical Development Co. Ltd, Hoddesdon, UK). These settings, determined from preliminary tests on an arbitrary sample of shoots, were kept constant for all measurements.

Root and shoot development

All cuttings were gently removed from the propagation mix at 35 d post-setting and assessed for mortality, rooting and number of primary roots (i.e. roots arising directly from the stem). Cuttings that had formed roots were transferred to water-filled glass jars and stored at 4°C in darkness for subsequent root and leaf scanning. Cuttings were prepared for root scanning by severing roots from the stem, gently washing the root system in tap water to remove propagation mix, and blotting the roots dry. Root systems were placed flat, scanned, and their images analysed using a WinRHIZO LA1600+ scanner with WinRHIZO PRO v.2004b image analysis software (Régent Instruments Inc, Ste-Foy, Canada). Total length, surface area and volume of roots were recorded for each cutting. Leaves were also blotted dry, scanned, and their areas analysed using WinFOLIA PRO v.2004a software. The shoot and root systems of each cutting were then placed in separate paper bags, dried for several days at 65°C, and weighed.

Statistical analyses

Root development, shoot development and mortality were analysed by 1-way ANOVA for each taxon because extensive 2-way and 3-way interactions were found between taxa, seasons and hormone treatments. Analyses of rooting percentage and mortality included all cuttings, but analyses of primary root number, root length, root surface area, root volume, root dry weight, leaf area, and shoot dry weight included only rooted cuttings. Chlorophyll fluorescence was analysed by 2-way ANOVA (hormone treatment × day) because significant interactions were not detected between these two factors for any taxon. Square root or arcsine square root transformations were applied when data was not normally distributed. Post-hoc least significant difference (LSD) tests were performed only when significant differences were detected by ANOVA. Means are reported with standard errors, and treatment differences or interactions were regarded as significant at $P < 0.05$.

RESULTS

Initial cutting size

Leaf area of *C. torelliana* cuttings was higher than that of *C. citriodora* cuttings, with hybrid cuttings clearly intermediate between the parental species in winter but not in spring (**Table 1**). Pruning removed approximately 50–60% of the leaf area for *C. torelliana* and hybrid cuttings, and slightly less than 50% for the taxon with the lowest initial leaf area, *C. citriodora*.

Chlorophyll fluorescence

IBA treatments, in most cases, did not significantly affect maximum photochemical efficiency (F_V/F_M) of cuttings' leaves, either at 0800 H or 1300 H (**Fig. 2**). However, the 8 g IBA / kg treatment did reduce F_V/F_M, both at 0800 H and 1300 H, of the hybrid in winter (**Fig. 2C**). In spring, the 1 g and 3 g IBA / kg treatments reduced F_V/F_M at 0800 H for *C. torelliana* (**Fig. 2B**) but increased F_V/F_M at 0800 H for the hybrid (**Fig. 2D**).

F_V/F_M at 0800 H was always high (approx. 0.80) on the day after setting and did not decline significantly by 15 d

Table 1 Initial leaf area (pre- and post-pruning) and shoot dry weight (post-pruning) of cuttings of *Corymbia torelliana*, *C. citriodora* and three hybrid families.

	Area pre-pruning (cm^2)	Area post-pruning (cm^2)	Area pruned (%)	Dry weight (mg)
Winter experiment				
C. torelliana	22.3 ± 2.8^a	9.0 ± 0.9^a	58.4 ± 1.4^a	105.7 ± 16.0^a
Hybrid 13	13.6 ± 1.3^b	5.5 ± 0.5^b	59.4 ± 1.1^a	67.0 ± 7.1^{bc}
Hybrid 16	15.8 ± 2.2^b	6.3 ± 0.8^b	60.0 ± 0.8^a	87.6 ± 15.8^{ab}
Hybrid 30	12.0 ± 1.3^b	5.2 ± 0.6^b	56.7 ± 2.3^a	53.5 ± 7.4^{cd}
C. citriodora	5.5 ± 0.7^c	2.9 ± 0.3^c	46.8 ± 2.2^b	29.4 ± 2.9^d
Spring experiment 2				
C. torelliana	14.1 ± 1.9^{ab}	5.6 ± 0.7^{ab}	59.9 ± 1.4^a	33.7 ± 5.8
Hybrid 13	11.9 ± 1.4^{ab}	5.3 ± 0.6^{abc}	54.6 ± 1.8^{abc}	35.0 ± 4.5
Hybrid 16	10.1 ± 2.0^{bc}	4.8 ± 0.9^{bc}	51.1 ± 1.7^{bc}	32.4 ± 6.7
Hybrid 30	15.8 ± 1.9^a	6.8 ± 0.8^a	56.1 ± 2.2^{ab}	40.0 ± 5.1
C. citriodora	6.6 ± 0.7^c	3.3 ± 0.2^c	49.1 ± 2.7^c	25.1 ± 2.2

Means (± SE) with different letters among the five taxa within an experiment are significantly different (ANOVA and LSD test, $P < 0.05$, $n = 10$ cuttings)
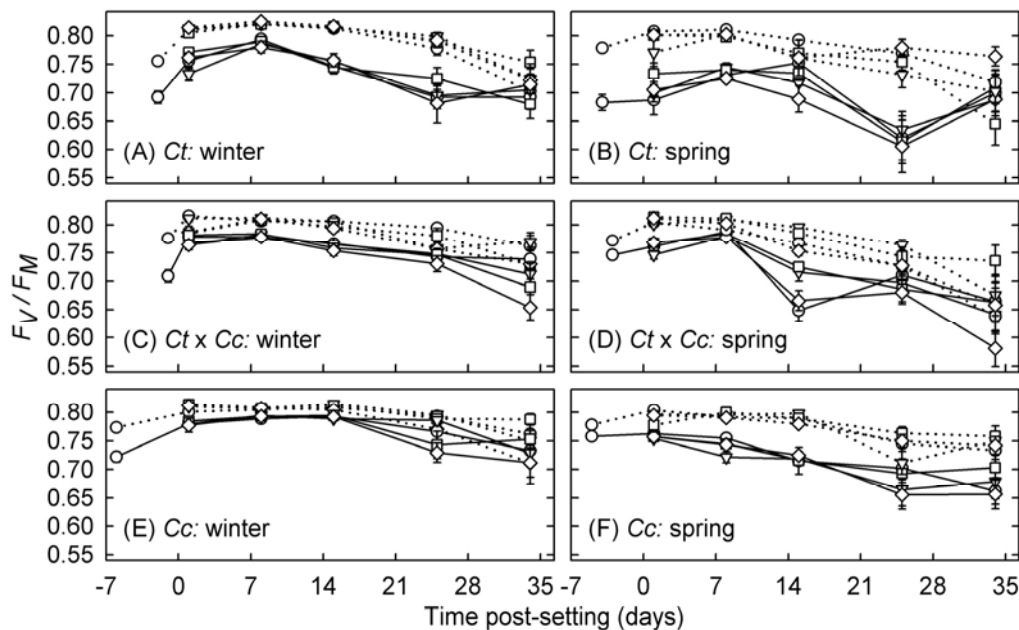
Fig. 2 Maximum photochemical efficiency (F_V/F_M) at 0800 hours (dotted lines) and 1300 hours (solid lines) for cuttings of (**A, B**) *C. torelliana* (*Ct*), (**C, D**) hybrid family 13 (*Ct × Cc*), and (**E, F**) *C. citriodora* (*Cc*) subjected to one of four levels of rooting hormone (○ 0; ▽ 1; □ 3; ◊ 8 g indole-3-butyric acid/kg powder) in winter or spring. Means are provided with standard errors (*n* = 20 pre-setting; *n* = 15 post-setting). Treatment effects are not significant in most cases (see text), but F_V/F_M is significantly lower at 25 d and 34 d post-setting than at 1 d and 8 d post-setting (2-way ANOVA and LSD test, *P* < 0.05) in all cases except for *C. torelliana* cuttings at 1300 H, 34 d post-setting in spring.
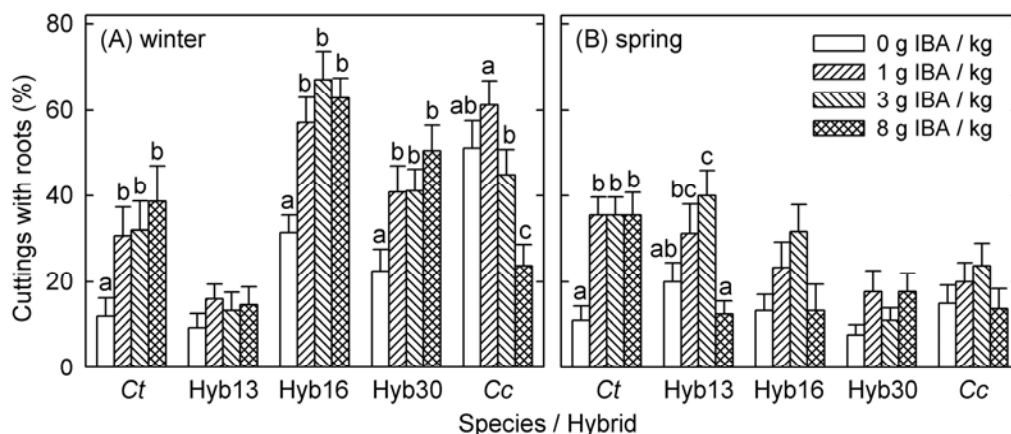

Fig. 3 Percentage of cuttings with roots for *C. torelliana* (*Ct*), three hybrid families (Hyb 13, Hyb 16 and Hyb 30) and *C. citriodora* (*Cc*) subjected to one of four levels of rooting hormone in (**A**) winter or (**B**) spring. Means (+ SE) with different letters within a species or hybrid family are significantly different (ANOVA and LSD test, *P* < 0.05, *n* = 12-18).

post-setting, except for slight reductions in spring for *C. torelliana* (**Fig. 2B**) and the hybrid (**Fig. 2D**). Similarly, F_V/F_M at 1300 H did not decline significantly between 1 d and 15 d post-setting, except in spring for the hybrid (**Fig. 2D**) and *C. citriodora* (**Fig. 2F**). In every case, F_V/F_M at both 0800 H and 1300 H fell significantly by 25 d post-setting. It did not recover by 34 d post-setting except in the case of *C. torelliana* cuttings at 1300 H in spring (**Fig. 2B**).

Root and shoot development

In contrast with chlorophyll fluorescence, the percentage of cuttings that formed roots was often affected by IBA application (**Fig. 3**). All IBA doses significantly increased rooting percentage for *C. torelliana*, both in winter and spring. Various IBA doses increased rooting percentage for the hybrid families, but only 3 g IBA/kg provided a significant effect in all three families, and this effect was only evident in one of two seasons for each family. In the five out of eight instances for *C. torelliana* and hybrid families where 3 g IBA/kg significantly improved rooting percentage, a further 18.6–35.7% of cuttings (in absolute terms) formed roots compared with the nil-hormone treatment,

equating to relative increases of 83–220% in rooted cuttings production.

IBA applications did not increase rooting percentages for *C. citriodora* cuttings (**Fig. 3**), but the 8 g IBA / kg treatment greatly reduced rooting percentage in winter. In this instance, 8 g IBA / kg significantly increased cutting mortality (**Fig. 4A**), as it did when applied to any of the taxa in spring (**Fig. 4B**). *C. citriodora* cuttings proved particularly susceptible to IBA, because even the lowest dose (1 g IBA/kg) increased cutting mortality in spring for this species.

Application of 8 g IBA/kg greatly elevated the number of primary roots per rooted cutting, except for hybrid family 13 in winter (**Fig. 5**). Mean numbers of primary roots for nil-hormone treated cuttings were between 1.1 ± 0.1 and 2.0 ± 0.4 but the means following application of 8 g IBA/kg ranged from 3.1 ± 0.4 to 5.3 ± 0.9 (excluding hybrid family 13 in winter). The intermediate dose, 3 g IBA/kg, was also effective for *C. torelliana* and hybrid family 16 in both seasons, *C. citriodora* in winter, and hybrid family 13 in spring. The range of means in these six cases was 3.4 ± 0.7 to 4.1 ± 0.5.
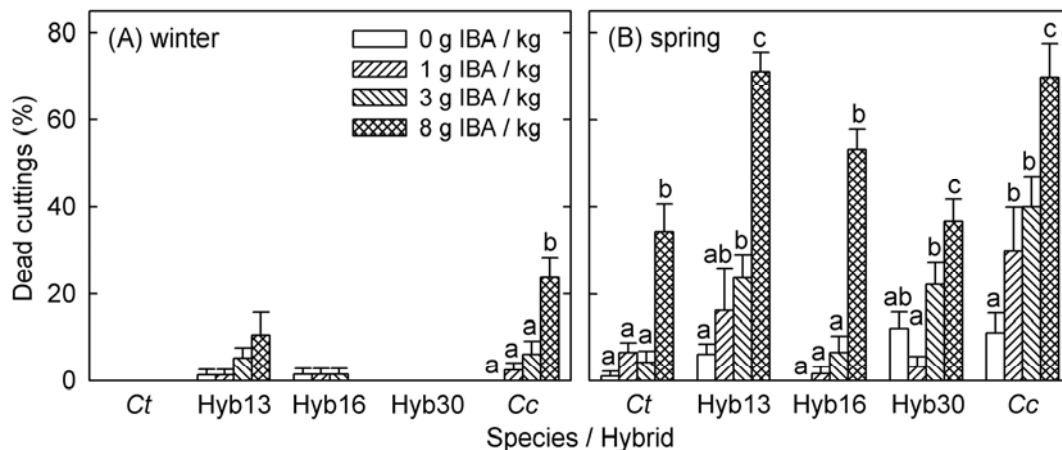
Despite the frequent marked effects of IBA application

Fig. 4 Percentage of dead cuttings for *C. torelliana* (*Ct*), three hybrid families (Hyb 13, Hyb 16 and Hyb 30) and *C. citriodora* (*Cc*) subjected to one of four levels of rooting hormone in (**A**) winter or (**B**) spring. Means (+ SE) with different letters within a species or hybrid family are significantly different (ANOVA and LSD test, *P* < 0.05, *n* = 12-18).
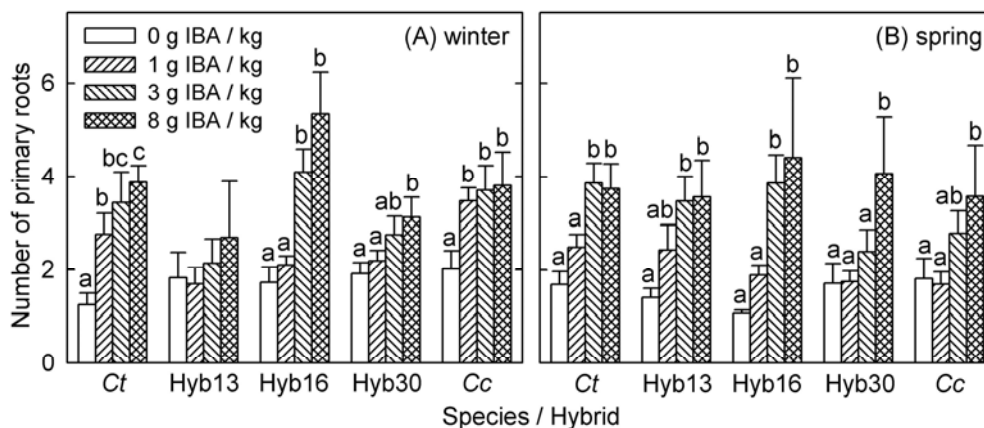

Fig. 5 Number of primary roots per rooted cutting for *C. torelliana* (*Ct*), three hybrid families (Hyb 13, Hyb 16 and Hyb 30) and *C. citriodora* (*Cc*) subjected to one of four levels of rooting hormone in (**A**) winter or (**B**) spring. Means (+ SE) with different letters within a species or hybrid family are significantly different (ANOVA and LSD test, *P* < 0.05, *n* = 4-18).

on primary root number of rooted cuttings, hormone treatments mostly had no significant effect on root volume (**Tables 2, 3**). Similarly, hormone treatments mostly had no significant effect on root length, root surface area or root dry weight (data not presented). Exceptions were in spring, when the 3 g and 8 g IBA/kg treatments, respectively, reduced root surface area (by 42 and 42%), root dry weight (by 51 and 54%) and root volume (by 50 and 49%) of *C. torelliana* cuttings and root volume (by 52 and 50%) of *C. citriodora* cuttings. Application of 1 g IBA/kg was also sufficient to reduce root dry weight (by 38%) and root volume (by 35%) of the *C. torelliana* cuttings.

Significant effects of IBA treatments on leaf area or shoot dry weight of rooted cuttings were more common, being evident in at least one season for all taxa except hybrid family 30 (**Tables 2, 3**). The 8 g IBA/kg treatment often caused leaf abscission, significantly reducing leaf area in four instances (by 55–79%) and shoot dry weight in four instances (by 40–58%). In addition, in spring (**Table 3**), the 1 g and 3 g IBA/kg treatments, respectively, reduced leaf area for *C. torelliana* (by 50 and 59%) and *C. citriodora* (by 58 and 67%), and reduced shoot dry weight for *C. torelliana* (by 40 and 54%).

DISCUSSION

Application of IBA had strongly promotive, albeit inconsistent, effects on the percentage of *C. torelliana* and hybrid cuttings that formed roots, with the intermediate dose increasing rooting percentages in both seasons for *C. torelliana* and in one of two seasons for each hybrid family. IBA application more consistently increased the number of primary roots per rooted cutting, not only for *C. torelliana* and

hybrids, but also for *C. citriodora*. Many nil-hormone treated cuttings produced just one or two roots but the high IBA dose greatly increased root number in nine cases out of ten. However, there was a trade-off in spring (and, to a lesser extent, in winter for *C. citriodora*) between inducing high root number and causing cutting mortality.

Despite its frequent marked effects on rooting percentage, primary root number and mortality of *Corymbia* cuttings, IBA application had little or no effect on photoinhibition. Cuttings experienced little photoinhibition for the first 8 or 15 days after severance from the stock plant and placement under mist irrigation. During this phase, maximum photochemical efficiency (F_V/F_M) of cuttings declined during the day but recovered overnight to approximately 0.80. Similarly-high F_V/F_M has been observed in cuttings of *Wollemia nobilis* during the first two weeks under intermittent mist (Pohio *et al.* 2005), whereas F_V/F_M fell rapidly for cuttings of *Cordia alliodora*, *Albizia guachapele* and *Taxus × media* in non-mist propagation systems (Mesén *et al.* 1997, 2001; Bruce *et al.* 2001). These results, in combination, suggest that mist irrigation effectively minimizes vapour pressure deficit and prevents rapid onset of leaf stress following severance of cuttings from the stock plant.

Photoinhibition of *Corymbia* cuttings became more evident by 25 days after severance. Mean afternoon F_V/F_M at 34 d post-setting ranged from 0.65–0.75 in winter and from 0.58–0.71 in spring, similar to final values reported for *Wollemia* cuttings under mist (Pohio *et al.* 2005) but less severe than photoinhibition experienced by cuttings of *Cordia* (0.12–0.60) and *Albizia* (0.45–0.59) in non-mist propagators (Mesén *et al.* 1997, 2001). Unrooted cuttings are prone to nutrient leaching, nutrient deficiency and photoinhibition under mist irrigation, because little uptake of nutrients oc-

Table 2 Root volume, leaf area and shoot dry weight of rooted cuttings of *Corymbia torelliana*, *C. citriodora* and three hybrid families subjected to one of four IBA levels (winter experiment).

	IBA concentration (g kg^{-1})			
	0	**1**	**3**	**8**
C. torelliana				
Root volume (cm^3)	0.10 ± 0.04	0.11 ± 0.03	0.17 ± 0.04	0.17 ± 0.02
Leaf area (cm^2)	15.3 ± 4.3	15.7 ± 3.6	14.0 ± 2.0	11.9 ± 1.7
Shoot weight (mg)	80.0 ± 6.8	84.5 ± 10.4	87.3 ± 9.8	79.0 ± 7.1
Hybrid 13				
Root volume (cm^3)	0.19 ± 0.03	0.16 ± 0.05	0.15 ± 0.07	0.10 ± 0.03
Leaf area (cm^2)	17.9 ± 6.9 a	13.0 ± 4.4 ab	11.6 ± 6.2 ab	3.8 ± 0.8 b
Shoot weight (mg)	70.0 ± 12.9 a	71.0 ± 14.9 a	47.1 ± 11.7 ab	33.8 ± 5.6 b
Hybrid 16				
Root volume (cm^3)	0.19 ± 0.04	0.15 ± 0.03	0.14 ± 0.02	0.14 ± 0.02
Leaf area (cm^2)	26.2 ± 8.0	20.3 ± 4.6	13.9 ± 2.5	13.0 ± 2.60
Shoot weight (mg)	116.2 ± 21.5 a	107.9 ± 12.7 ab	75.7 ± 9.1bc	70.0 ± 11.8 c
Hybrid 30				
Root volume (cm^3)	0.20 ± 0.05	0.19 ± 0.03	0.19 ± 0.04	0.17 ± 0.02
Leaf area (cm^2)	20.6 ± 4.6	19.7 ± 3.6	18.0 ± 3.2	15.0 ± 3.50
Shoot weight (mg)	87.3 ± 14.7	97.6 ± 7.2	88.2 ± 9.0	70.6 ± 9.60
C. citriodora				
Root volume (cm^3)	0.11 ± 0.02	0.18 ± 0.04	0.14 ± 0.03	0.11 ± 0.03
Leaf area (cm^2)	8.4 ± 1.1 a	16.1 ± 5.0 a	13.0 ± 3.3 a	3.8 ± 2.1 b
Shoot weight (mg)	65.3 ± 7.3 a	79.4 ± 13.6 a	69.4 ± 9.9 a	28.3 ± 7.4 b

Means (± SE) with different letters within a species or hybrid family are significantly different (ANOVA and LSD test, $P < 0.05$, n = 6-17).

Table 3 Root volume, leaf area and shoot dry weight of rooted cuttings of *Corymbia torelliana*, *C. citriodora* and three hybrid families subjected to one of four IBA levels (spring experiment).

	IBA concentration (g kg^{-1})			
	0	**1**	**3**	**8**
C. torelliana				
Root volume (cm^3)	0.45 ± 0.13 a	0.29 ± 0.05 b	0.22 ± 0.03 b	0.23 ± 0.03 b
Leaf area (cm^2)	57.9 ± 16.7 a	28.8 ± 6.4 b	23.5 ± 4.2 b	23.4 ± 5.1 b
Shoot weight (mg)	211.2 ± 54.8 a	126.4 ± 20.7 b	97.6 ± 13.1 b	89.3 ± 12.6 b
Hybrid 13				
Root volume (cm^3)	0.15 ± 0.03	0.24 ± 0.06	0.24 ± 0.04	0.18 ± 0.04
Leaf area (cm^2)	10.6 ± 2.5	28.2 ± 7.6	24.0 ± 6.1	13.2 ± 2.7
Shoot weight (mg)	66.8 ± 9.3	137.3 ± 31.1	111.1 ± 23.2	88.5 ± 15.7
Hybrid 16				
Root volume (cm^3)	0.33 ± 0.12	0.28 ± 0.11	0.27 ± 0.07	0.25 ± 0.14
Leaf area (cm^2)	31.9 ± 14.8	24.4 ± 10.1	20.7 ± 5.2	8.9 ± 5.1
Shoot weight (mg)	128.8 ± 38.4	106.9 ± 32.4	81.4 ± 14.3	53.4 ± 21.5
Hybrid 30				
Root volume (cm^3)	0.31 ± 0.11	0.33 ± 0.10	0.37 ± 0.11	0.29 ± 0.07
Leaf area (cm^2)	35.7 ± 16.2	48.0 ± 12.8	43.9 ± 11.8	23.8 ± 10.5
Shoot weight (mg)	146.8 ± 51.0	178.3 ± 40.2	176.2 ± 38.9	101.7 ± 31.3
C. citriodora				
Root volume (cm^3)	0.37 ± 0.11 a	0.21 ± 0.03 ab	0.18 ± 0.02 b	0.19 ± 0.06 b
Leaf area (cm^2)	28.4 ± 11.6 a	11.8 ± 4.3 b	9.3 ± 1.9 b	10.6 ± 3.9 b
Shoot weight (mg)	133.7 ± 44.9	79.7 ± 18.5	65.3 ± 7.8	69.0 ± 17.5

Means (± SE) with different letters within a species are significantly different (ANOVA and LSD test, $P < 0.05$, n = 4-18)

curs through the severed base of cuttings (Grange and Loach 1983; Blazich 1988b; Hartmann *et al.* 1997) and nitrogen deficiency can predispose leaves to photoinhibition (Skillman and Osmond 1998; Fernandes *et al.* 2002). Recovery of F_V/F_M upon formation of roots has been reported for cuttings of *Taxus* and *Wollemia* (Bruce *et al.* 2001; Pohio *et al.* 2005). For *Corymbia*, a recovery of F_V/F_M by 34 days post-setting was only evident in the case of afternoon values for *C. torelliana* cuttings in spring. A notable difference between the current and previous studies (Bruce *et al.* 2001; Pohio *et al.* 2005) was that final rooting percentages for *Corymbia* (9–67%) were much lower than for *Taxus* (32–97%) and *Wollemia* (65–85%). Therefore, greater proportions of *Corymbia* cuttings were in physiological decline, rather than recovery, at the conclusion of experiments.

No consistent relationship emerged between photoinhibition, rooting and mortality among the few cases where IBA affected photoinhibition of *Corymbia* cuttings. Importantly, lower F_V/F_M was never associated with lower rooting or higher mortality. Lethal effects of high IBA doses on *Corymbia* cuttings were not the result of increased photoin-

hibition but were caused by other factors, possibly including ethylene production. Exogenous auxin stimulates ethylene synthesis in many species (Mudge 1988; Taylor and Whitelaw 2001) and leaf abscission is associated with ethylene production (Bangerth 2000; Taylor and Whitelaw 2001). In *Corymbia*, the high IBA dose caused extensive abscission of green and apparently-healthy leaves, and significantly reduced final leaf area or shoot dry weight of rooted cuttings (by 40–79%) in at least one season for most taxa. These effects on shoot system development were associated with a halving of root volume for *C. torelliana* and *C. citriodora* rooted cuttings in spring, even though the high IBA dose induced much higher numbers of primary roots.

An ultimate aim of the study was to develop a vegetative propagation system for plantation establishment of *C. torelliana* × *C. citriodora* rooted cuttings. The intermediate auxin dose (3 g IBA/kg powder) was clearly the most consistent in raising both rooting percentages and root numbers, whilst having little or no effect on mortality, leaf area or shoot weight, of hybrid cuttings. Combining results across both seasons for each hybrid family, the intermediate dose

increased total numbers of rooted cuttings by 72–121%. Similar increases have been observed following IBA treatment of *Eucalyptus grandis* and its hybrids (Wendling *et al.* 2000; Zuffellato-Ribas and Rodrigues 2001), *E. globulus* and *E. saligna* (Corrêa *et al.* 2005; Fogaça and Fett-Neto 2005).

The intermediate IBA dose also increased primary root number in one of two seasons for each hybrid family, from $1.1 \pm 0.1 - 1.7 \pm 0.3$ (nil hormone) to $3.5 \pm 0.5 - 4.1 \pm 0.5$ (3 g IBA/kg powder). Similar effects have been observed in other eucalypts that produce just one or two primary roots per nil-hormone treated cutting (Corrêa and Fett-Neto 2004; Corrêa *et al.* 2005; Fogaça and Fett-Neto 2005). Because nil-hormone treated eucalypt cuttings produce so few roots, there appears ample scope for auxin application to improve root system symmetry, and thus tree stability (Coutts *et al.* 1999; Khuder *et al.* 2007). Other measures of field performance such as tree survival, height and stem diameter are often positively, though weakly, related to primary root number. For example, raising primary root number from 1 to 5 has resulted in an 11% increase in nursery survival (Goldfarb *et al.* 1998), a 23% increase in tree height after 2 years (Haines *et al.* 1992) and a 12% increase in stem diameter after 5 years (Foster *et al.* 2000) for various *Pinus* taxa.

In conclusion, IBA application had little or no effect on photoinhibition of *Corymbia* cuttings but it had frequent, strong, dose-dependent effects on root formation and cutting mortality. The high IBA dose, in particular, greatly elevated number of primary roots per rooted cutting, but also caused extensive cutting mortality in spring. Increases in primary root number did not result in higher root volume, weight, surface area or length, possibly because high IBA also tended to cause leaf abscission and reduced shoot weight. The most effective treatment for multiplication of *Corymbia* hybrids was the intermediate dose (3 g IBA/kg powder), which most consistently elevated rooting percentages and root numbers whilst having little or no effect on mortality, leaf area or shoot weight. This treatment showed capacity, on average, to double the percentage of hybrid cuttings that formed roots in each setting. Such increases can have enormous effects on final numbers of field plants, because each rooted cutting can develop into a stock plant that, in turn, produces more rooted cuttings and then more stock plants (Trueman *et al.* 2007). Large-scale deployment of *Corymbia* hybrids is therefore possible using a vegetative propagation system to multiply superior individuals or selected families, but further improvements to optimum-treatment rooting percentages across a range of families (currently 11–67%) are required to encourage production of a genetically diverse range of hybrids by commercial nurseries.

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