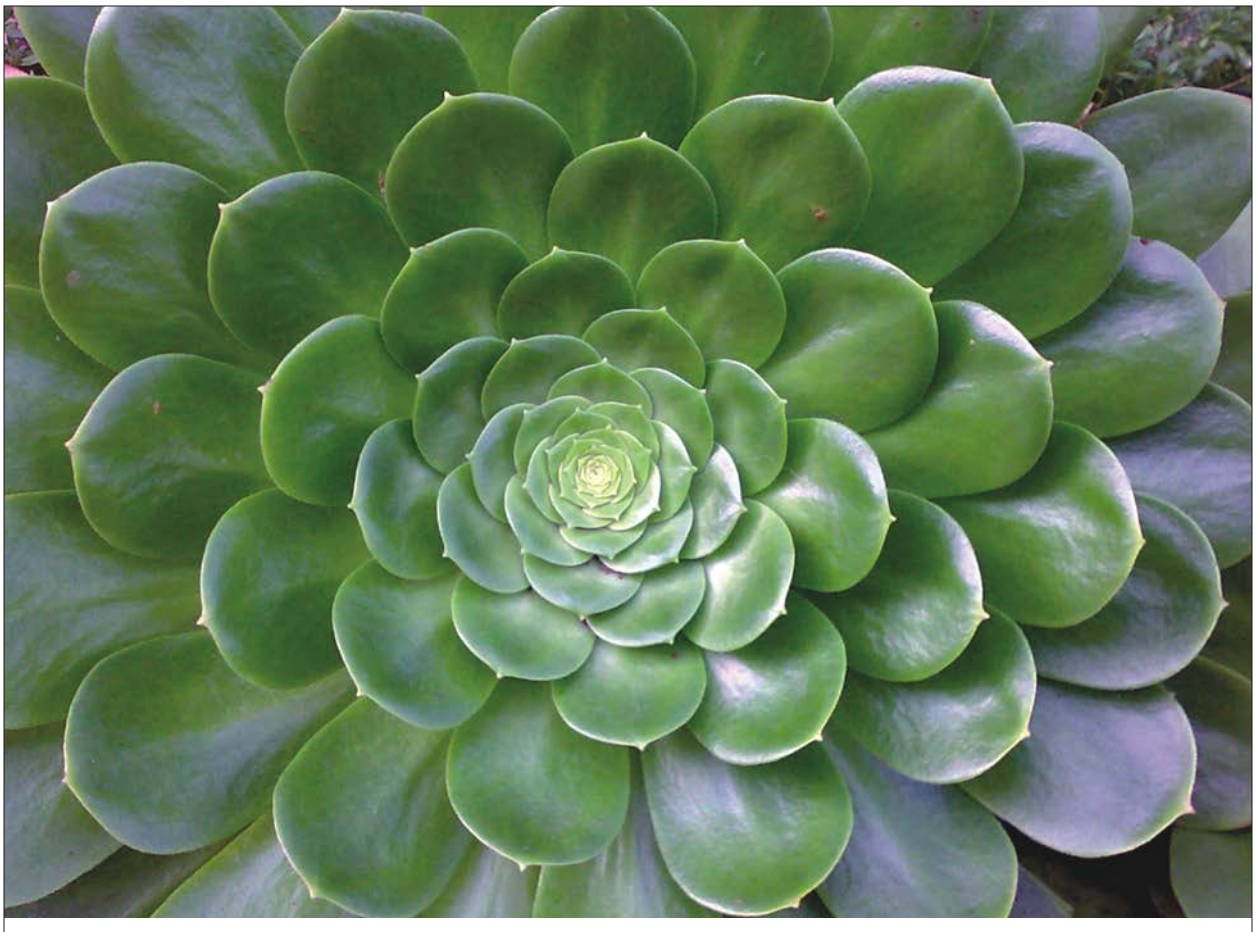

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Improved Root Formation in Eucalypt Cuttings Following Combined Auxin and Anti-ethylene Treatments

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ABSTRACT

Improved vegetative propagation methods are needed for eucalypt hybrids because demand for eucalypt hybrids is often greater than their seed supply and it can be difficult to produce adventitious roots on eucalypt cuttings. This study examined the timing of adventitious root formation in cuttings of two eucalypt hybrids, *Corymbia torelliana*×*Corymbia citriodora* and *Eucalyptus pellita*×*Eucalyptus grandis* and determined the effects of combining Indole-3-butyric Acid (IBA) with an ethylene inhibitor, 1-methylcyclopropene (MCP) or aminoethoxyvinylglycine (AVG), on rooting, defoliation and death of cuttings. Root initiation commenced within 14 days, well before the main phases of cutting defoliation and death. IBA often increased rooting percentage or the number of adventitious roots per rooted cutting. IBA also increased defoliation and death of *Corymbia* cuttings in one experiment, but AVG alleviated these effects and increased the percentage of cuttings that formed roots. The combined IBA and MCP or AVG treatments frequently increased cutting production and root system quality. Combining IBA (8 g kg^{-1}) with MCP (400 nl L^{-1}) or AVG (125 mg L^{-1}) raised the number of *Corymbia* rooted cuttings by 83 and 206%, respectively and the number of *Eucalyptus* rooted cuttings by 46 and 110%, respectively. These *Corymbia* plants possessed 2.2 and 2.6 more adventitious roots and the *Eucalyptus* plants possessed 1.0 and 1.1 more adventitious roots, on average, than untreated cuttings. The rooting percentages obtained with optimal treatments (30-42 and 29-59% for *Corymbia* and *Eucalyptus*, respectively) allow hybrid deployment through clonal propagation. The combination of an auxin rooting hormone with an ethylene inhibitor is a novel and effective treatment for improving vegetative propagation of eucalypt hybrids.

Key words: Abscission, *Eucalyptus*, propagation, rooting, senescence

INTRODUCTION

The eucalypts, *Eucalyptus*, *Corymbia* and *Angophora*, are the world's most widely planted hardwood trees because of their broad diversity of species, adaptability to a wide range of soils and climate, fast growth and exceptional wood qualities (Teulieres *et al*., 2007; Nichols *et al*., 2010). Most eucalypt plantations are located in tropical and subtropical regions and, increasingly, eucalypt species and hybrids are being introduced into marginal rainfall areas (Teulieres *et al*., 2007; Lee *et al*., 2009). One hybrid showing promise in marginal rainfall areas of subtropical Australia

is *Corymbia torelliana*×*C. citriodora* which combines frost and disease tolerance of *C. torelliana* with the wood properties of *C. citriodora* (Lee, 2007; Lee *et al*., 2009). The preferred eucalypt for tropical regions in northern Australia is *Eucalyptus pellita* (Brawner *et al*., 2010) and there is increasing interest among plantation growers in *E. pellita* hybrids such as *E. pellita*×*E. grandis*.

Supply of seed is a major constraint in establishing plantations of eucalypt hybrids. Seed supply can be limited by sparse and irregular flowering (Lee, 2007; Lee *et al*., 2009) and the high expense associated with manual pollination and seed collection in the tree canopy (Dickinson *et al*., 2010). These limitations can be overcome using a strategy of 'clonal forestry' or 'vegetative family forestry', whereby plantation trees are produced from cuttings harvested from a limited supply of stock plants derived from seedlings (Eldridge *et al*., 1994; Trueman, 2006; Lee, 2007; Hung and Trueman, 2010). Although, some eucalypts such as *Eucalyptus deglupta*, *Eucalyptus grandis* and *Eucalyptus saligna* are easy to propagate from cuttings (Eldridge *et al*., 1994; Fogaca and Fett-Neto, 2005; Wendling and Xavier, 2005), most plantation eucalypts including *C. citriodora*, *C. torelliana*, *E. cloeziana*, *E. dunnii*, *E. globulus* and *E. nitens* are very difficult to propagate (Luckman and Menary, 2002; Assis *et al*., 2004; Fogaca and Fett-Neto, 2005; Smith and Henson, 2007; Trueman and Richardson, 2008).

Application of auxin, in particular indole-3-butyric acid (IBA), is one of the most common treatments to enhance rooting of cuttings (Hartmann *et al*., 1997; Leakey, 2004). IBA is used on many tree species including eucalypts (Wendling *et al*., 2000; Zuffellato-Ribas and Rodrigues, 2001; Fogaca and Fett-Neto, 2005) to increase the percentage of cuttings that forms roots and the number of adventitious roots per cutting, to accelerate root initiation and to improve root system quality and uniformity (Leakey, 2004; Hunt *et al*., 2011; Husen, 2012). However, some species and hybrids appear unresponsive to auxin (Wendling and Xavier, 2005; Trueman and Peters, 2006; Trueman *et al*., 2012) and high doses can cause cutting death (Perry and Trueman, 1999; Zuffellato-Ribas and Rodrigues, 2001; Wendling and Xavier, 2005). High IBA doses reduce rooting and increase defoliation and death of *C. citriodora*, *C. henryi*, *C. torelliana* and *C. citriodora*×*C. torelliana* cuttings (Catesby and Walker, 1998; Trueman and Richardson, 2008). The mechanism of IBA-induced defoliation and death remains unknown, although IBA does not reduce chlorophyll fluorescence of the leaves of cuttings (Trueman and Richardson, 2008, 2011).

Leaf senescence and abscission are promoted by accumulation of another hormone, ethylene (Abeles *et al*., 1992; Kadner and Druege, 2004). Auxin application stimulates ethylene production (Sun and Bassuk, 1993; Zhu *et al*., 2008) and so auxin-induced ethylene accumulation may be the primary cause of defoliation in eucalypt cuttings. The ethylene perception inhibitor, 1-methylcyclopropene (MCP) and ethylene synthesis inhibitor, Aminoethoxyvinylglycine (AVG), are used commercially to inhibit ethylene responses and prevent or delay fruit ripening, fruit abscission or leaf abscission (Adkins *et al*., 2005; Yuan and Carbaugh, 2007; Yuan and Li, 2008). Neither ethylene inhibitor has been tested on woody plant cuttings. The ethylene perception inhibitor, Silver Thiosulphate (STS), increases root formation in mung bean cuttings when applied in combination with a high dose (100 μM) of Naphthaleneacetic Acid (NAA), but it inhibits root formation at a low dose (30 μM) of NAA (De Klerk and Hanecakova, 2008). This suggests that the amount of ethylene produced at the low NAA dose is beneficial for root formation whereas the high NAA dose stimulates supra-optimal ethylene production, thus inhibiting root formation. Roots are induced on micropropagated shoots of *C. maculata* when STS is added to IBA-containing medium (Steinitz *et al*., 2010), indicating that ethylene generated in the presence of IBA prevents adventitious root induction. IBA-induced ethylene production may also be the cause of defoliation and death in eucalypt cuttings, in which case the deleterious effects of high IBA doses could be prevented by ethylene inhibitors such as MCP, AVG or STS. The optimal time to apply ethylene

inhibitors may depend on the timing of root initiation because some ethylene can be beneficial during the initiation phase of root formation (De Klerk and Hanecakova, 2008; Mendes *et al.*, 2011).

The objectives of this study were to determine: (1) the relationships between IBA dose, the percentage of cuttings that forms roots and the number of adventitious roots per rooted cutting of *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis*, (2) the effect of IBA concentration on defoliation and death of cuttings, (3) how defoliation and death of cuttings is affected by combining the ethylene perception inhibitor, MCP or the ethylene synthesis inhibitor, AVG, with the IBA treatment, (4) how the percentage of cuttings with roots and the number of adventitious roots are affected by the combined IBA and MCP or AVG treatments and (5) the timing of adventitious root initiation and how this timing relates to the timing of defoliation and death of cuttings. These results will assist in developing improved techniques for clonal propagation of eucalypts and other woody plants.

MATERIALS AND METHODS

Stock plants: Seeds were obtained from the Hardwood Tree Improvement Group, Agri-Science Queensland, Gympie, Australia and sown in Oct 2009. The *C. torelliana*×*C. citriodora* seed lot comprised equal weights of seeds from each of nine full-sibling families produced by controlled pollination of individual trees near Gympie (26°11'S, 152°40'E). The *E. pellita*×*E. grandis* seed lot comprised equal weights of two bulk control-pollinated seedlots from Cairns (16°55'S, 145°45'E) and Walkamin (17°08'S, 145°25'E).

Seeds were sown in potting mix with a thin layer of vermiculite and germinated under mist irrigation (10 sec duration at 10 min intervals from 0600-1800 h and 10 sec duration at 20 min intervals from 18:00-06:00 h) in a glasshouse in Gympie. In Dec., 2009, 150 seedlings of each hybrid were carefully removed from the potting mix and transferred to 2.8-L pots containing a 75/25 (v/v) mixture of shredded pine bark and perlite with 3 kg of 8-9 month slow-release Osmocote™ fertiliser (Scotts International, Heerlen, The Netherlands), 3 kg lime (Unimin, Lilydale, Australia), 1 kg Micromax® micronutrients (Scotts Australia, Baulkham Hills, Australia), 1 kg Hydroflow™ wetting agent (Scotts Australia, Baulkham Hills, Australia) and 1 kg of gypsum incorporated per m^3. The seedlings were transferred to an adjacent translucent-white polyethylene chamber, with misting provided for 15 sec duration at 30 min intervals from 06:00-18:00 h but no watering provided from 1800-0600 h. Seedlings were pruned to approximately 30 cm height in Jan 2010 and maintained as hedged stock plants between 30 and 50 cm height by regular pruning. Each stock plant was provided with 150 mL of foliar fertiliser, containing 18 g L^{-1} Flowfeed GF9 (Growforce, Acacia Ridge, Australia), 3 mL L^{-1} Firmrite (Spraygro Liquid Fertilizers, Gillman, Australia) and 500 mg L^{-1} $MgSO_4$, 7 days prior to each harvest of cuttings.

General methods: Cuttings, comprising the 4 cm single-node segments of vertically oriented shoots, were harvested from the stock plants on 1 Mar. (Experiment 1) and 19 Apr., 2010 (Experiment 2). On each occasion, shoots from within each hybrid were mixed randomly and then dissected into cuttings and the cuttings were pruned by removing approximately 60% of their leaf length. A total of 1350 cuttings from each hybrid on each occasion were allocated randomly to nine treatments (Experiments 1 and 2, below). Each experiment comprised 90 trays of cuttings, with ten replicate trays of each of the nine treatments. Each tray contained one replicate of 15 *C. torelliana*×*C. citriodora* cuttings and one replicate of 15 *E. pellita*×*E. grandis* cuttings.

Each cutting was dipped 0.5 cm into treatment powder for 1 sec and placed 1 cm deep into a 90 mL propagation tube containing a 75/25 (v/v) mixture of perlite and shredded pine bark with

3 kg of 8-9 month slow-release Osmocote™ fertiliser and 1 kg of gypsum incorporated m^{-3} (Trueman and Richardson, 2008). In each experiment, cuttings in three treatments (i.e., 30 trays) were dipped in talcum powder containing no IBA, cuttings in another three treatments were dipped in powder containing 3 g kg^{-1} IBA and cuttings in the remaining three treatments were dipped in powder containing 8 g kg^{-1} IBA (Trueman and Richardson, 2008). The MCP or AVG component of each of the nine treatment combinations was applied subsequently (Experiments 1 and 2).

The trays were placed randomly under mist irrigation in a translucent-white polyethylene chamber at the University of the Sunshine Coast (26°72'S, 153°06'E) for Experiment 1 and in a glasshouse at Agri-Science Queensland, Gympie (26°11'S, 152°40'E), for Experiment 2. The large number of cuttings in each experiment did not allow both experiments to be conducted at the same location. Light mist irrigation at the University of the Sunshine Coast was provided for 60 sec duration at 5 min intervals (day and night), whereas heavier mist irrigation at Gympie was provided for 10 sec duration at 10 min intervals (06:00-18:00 h) and 10 sec duration at 20 min intervals (18:00-06:00 h). Temperatures were recorded for the duration of experiments using Tinytalk dataloggers (RS Components, Smithfield, Australia). Irradiance was measured hourly on two cloudless days, 26 Apr. 2010 at the University of the Sunshine Coast and 31 May 2010 at Gympie. At each time point, irradiance was measured at four positions within the chamber or glasshouse using a quantum sensor (Delta-T Devices Ltd., Cambridge, UK).

Experiment 1: MCP application: The 30 trays within each of the three IBA concentrations (0, 3 and 8 g kg^{-1}) were separated randomly 8 days after setting into three treatments, each with ten trays to be treated with one of three MCP concentrations: 0, 400 and 800 nl L^{-1} (Jiang *et al*., 1999; Kadner and Druege, 2004; Mahajan *et al*., 2010). This timing (8 days after setting) was determined from preliminary experiments which found no significant effects of time of application (0, 4 and 8 days) of MCP or AVG on root formation or cutting growth. Trays were placed into 80 L plastic tubs adjacent to the propagation chamber and MCP tablets were placed into activator vials (Rohm and Hass Co., Philadelphia, USA) to release gaseous MCP. The same method was used for the three treatments receiving no MCP, except that MCP tablets were not placed into the activator solution vials. The tubs were immediately closed and the lids were water-sealed to prevent loss of MCP and to avoid desiccation of cuttings. The trays of cuttings were left in the tubs overnight (17:00-07:00 h), the tubs were then opened and the trays were returned to the propagation chamber.

Experiment 2: AVG application: The 30 trays within each IBA concentration were separated randomly 8 days after setting into their three treatments, each with ten trays to be treated with one of three AVG concentrations: 0, 125 and 250 mg L^{-1} (Yuan and Carbaugh, 2007; Yuan and Li, 2008; Zhu *et al*., 2008). The trays were placed into 80 L plastic tubs adjacent to the glasshouse and the cuttings were sprayed with treatment solution until runoff. The tubs were immediately closed and the lids were water-sealed to prevent desiccation of cuttings. The trays of cuttings were left in the tubs overnight (17:00-07:00 h) to allow AVG uptake before the cuttings were returned to the glasshouse.

Defoliation, death and adventitious root formation: The numbers of defoliated cuttings and dead cuttings in each replicate were recorded every 7 days from 0-56 days after setting. Defoliation was defined as abscission of the original leaf on the cutting rather than abscission of newly-formed leaves on axillary shoots. All cuttings were carefully removed from the propagation tubes at 56 days after setting and the number of adventitious roots (i.e., roots arising directly from the stem) on each

cutting was recorded. The percentage of cuttings forming roots and the mean number of adventitious roots per rooted cutting were then calculated for each replicate.

Timing of adventitious root formation: An additional 30 cuttings of each hybrid from Experiment 1 (above) were dipped in talcum powder containing either no IBA (15 cuttings) or 8 g kg^{-1} IBA (15 cuttings). Five *C. torelliana*×*C. citriodora* and five *E. pellita*×*E. grandis* cuttings from each treatment were sampled randomly at 7, 14 and 21 days after setting and a 5 mm-long basal transverse section of each cutting was excised and fixed in a solution of 3% glutaraldehyde and 0.1 M phosphate buffer. Samples were washed in deionised water and 0.1 M phosphate buffer, dehydrated in an ascending Tertiary Butanol/ethanol (TBE) series (70, 85, 95, 100% TBE) and mounted in paraffin. They were transverse sectioned at 3-4 μm using a UYD-335 Automated Microtome (ProSciTech, Thuringowa, Australia), dewaxed with xylene and ethylene, stained with safranin and fast green (Pohio *et al*., 2005) and mounted with Permount mounting medium (ProSciTech, Thuringowa, Australia). All sections were examined for adventitious root formation using an Eclipse E200 microscope (Nikon, Lidcombe, Australia).

Statistical analyses: The final proportions of defoliated cuttings, dead cuttings and cuttings with roots and the numbers of adventitious roots per rooted cutting, were analysed by one-way ANOVA for each hybrid. Proportions were arcsine square root transformed and root numbers were square root transformed, when variance was heterogeneous. Duncan's multiple range tests were performed when significant differences were detected by ANOVA at $p<0.05$. In addition, the final proportions of defoliated cuttings, dead cuttings and cuttings with roots and the numbers of adventitious roots per rooted cutting were compared by t-tests, as a baseline comparison for untreated cuttings only, between the two experiments. Means are reported with standard errors.

RESULTS

Experiment 1: MCP application: Temperatures ranged from 14.8-37.7°C during the 8 week experimental period (Fig. 1a) and irradiance reached 0.535 mmol m^{-2} sec^{-1} on a cloudless day (Fig. 1b) in the polyethylene propagation chamber. IBA and MCP treatments did not significantly affect the percentage of cuttings with abscised leaves (Fig. 1c, d) or the percentage of dead cuttings (Fig. 1e, f) for either hybrid. Leaf abscission generally preceded death in both *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis* cuttings. Abscission was greatest between 3 and 6 weeks after setting for *C. torelliana*×*C. citriodora* (Fig. 1c) and between 3 and 5 weeks after setting for *E. pellita*×*E. grandis* (Fig. 1d). Mortality was highest in the eighth week after setting for both hybrids (Fig. 1e, f).

Some of the combined IBA/MCP treatments increased the percentage of cuttings that produced roots compared with untreated cuttings (Fig. 2a, b). The percentage of *C. torelliana*×*C. citriodora* cuttings that formed roots was increased when the cuttings were treated with 3 g kg^{-1} IBA and 400 or 800 nl L^{-1} MCP or with 8 g kg^{-1} IBA and 400 nl L^{-1} MCP (Fig. 2a). However, no significant differences in the percentages of cuttings with roots were found between MCP concentrations within any IBA concentration and there were no significant differences among IBA treatments in the absence of MCP (Fig. 2a). For *E. pellita*×*E. grandis*, 3 g kg^{-1} IBA combined with 400 or 800 nl L^{-1} MCP and all combinations of 8 g kg^{-1} IBA with or without MCP significantly increased the percentage of cuttings with roots compared with untreated cuttings (Fig. 2b).

Some of the combined IBA/MCP treatments significantly increased the number of adventitious roots per rooted cutting (Fig. 2c, d). The treatments, 8 g kg^{-1} IBA combined with 400 or 800 nl L^{-1}

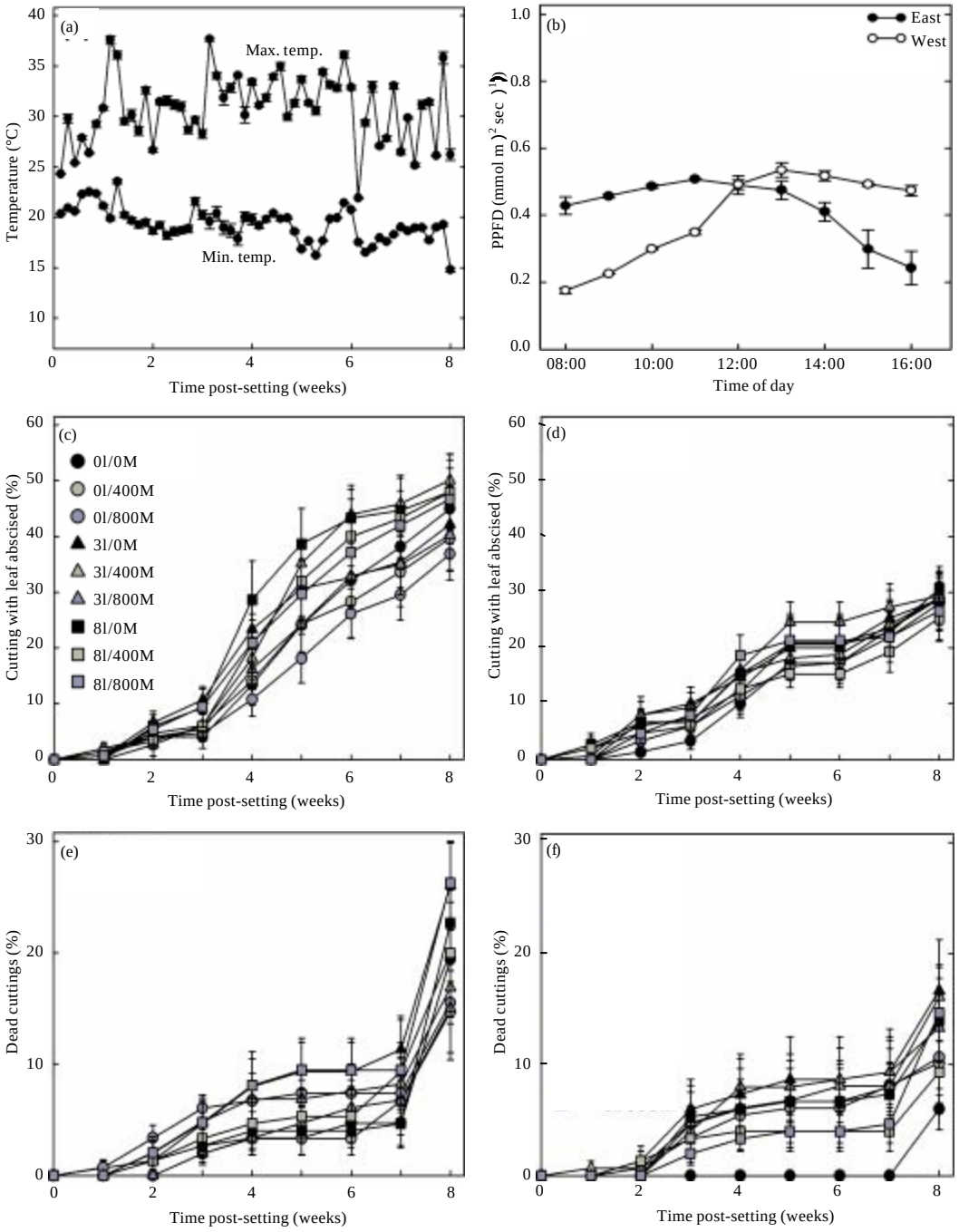

Fig. 1(a-f): Experiment 1: (a) Daily maximum and minimum temperatures and (b) Photosynthetic photon flux densities (PPFD) in the propagation chamber, Percentage of cuttings with the leaf abscised of (c) *Corymbia torelliana*×*C. citriodora* (Ct×Cc) and (d) *Eucalyptus pellita*×*E. grandis* (Ep×Eg) and mortality rate of (e) Ct×Cc and (f) Ep×Eg cuttings subjected to different concentrations of IBA (I, g kg^{-1}) and MCP (M, nl L^{-1}), Final Mean±SE within a hybrid do not differ significantly (ANOVA, $p>0.05$, n = 10)

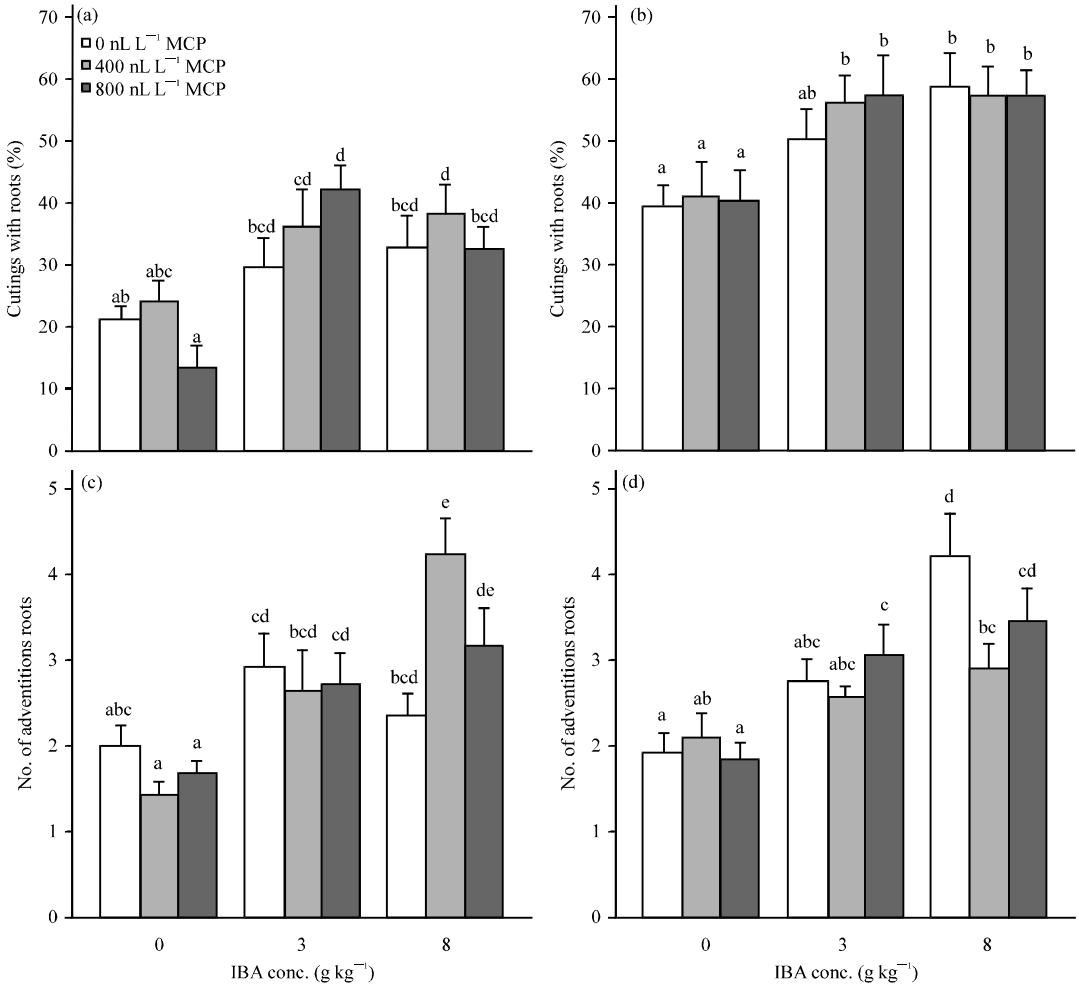

Fig. 2(a-d): Experiment 1: Percentage of cuttings with roots of (a) *Corymbia torelliana*×*C. citriodora* (Ct×Cc) and (b)*Eucalyptus pellita*×*E. grandis* (Ep×Eg) and Number of adventitious roots per rooted cutting of (c) Ct×Cc and (d) Ep×Eg cuttings subjected to different concentrations of IBA and MCP, Mean±SE with different letters within a hybrid are significantly different (ANOVA and Duncan's multiple range test, $p<0.05$, n = 10)

MCP, significantly increased the number of adventitious roots from *C. torelliana*×*C. citriodora* cuttings (Fig. 2c). The number of adventitious roots per rooted *E. pellita*×*E. grandis* cutting was significantly higher when the cuttings were treated with 3 g kg^{-1} IBA and 800 nl L^{-1} MCP or with 8 g kg^{-1} IBA with or without MCP than when the cuttings were untreated (Fig. 2d). In addition, significantly more adventitious roots were formed on *E. pellita*×*E. grandis* cuttings when the cuttings were treated with 8 g kg^{-1} IBA than when they were treated with 3 g kg^{-1} IBA (Fig. 2d).

Experiment 2: AVG concentration: Temperatures ranged from 14.6-31.2°C during the 8 week experimental period (Fig. 3a) and irradiance reached 1.133 mmol m^{-2} sec^{-1} on a cloudless day (Fig. 3b) in the glasshouse. More *C. torelliana*×*C. citriodora* cuttings displayed leaf abscission when treated with 3 or 8 g kg^{-1} IBA without AVG, when compared with untreated cuttings (Fig. 3c). This abscission was alleviated significantly by the application of 125 or 250 mg L^{-1} AVG (Fig. 3c). Two

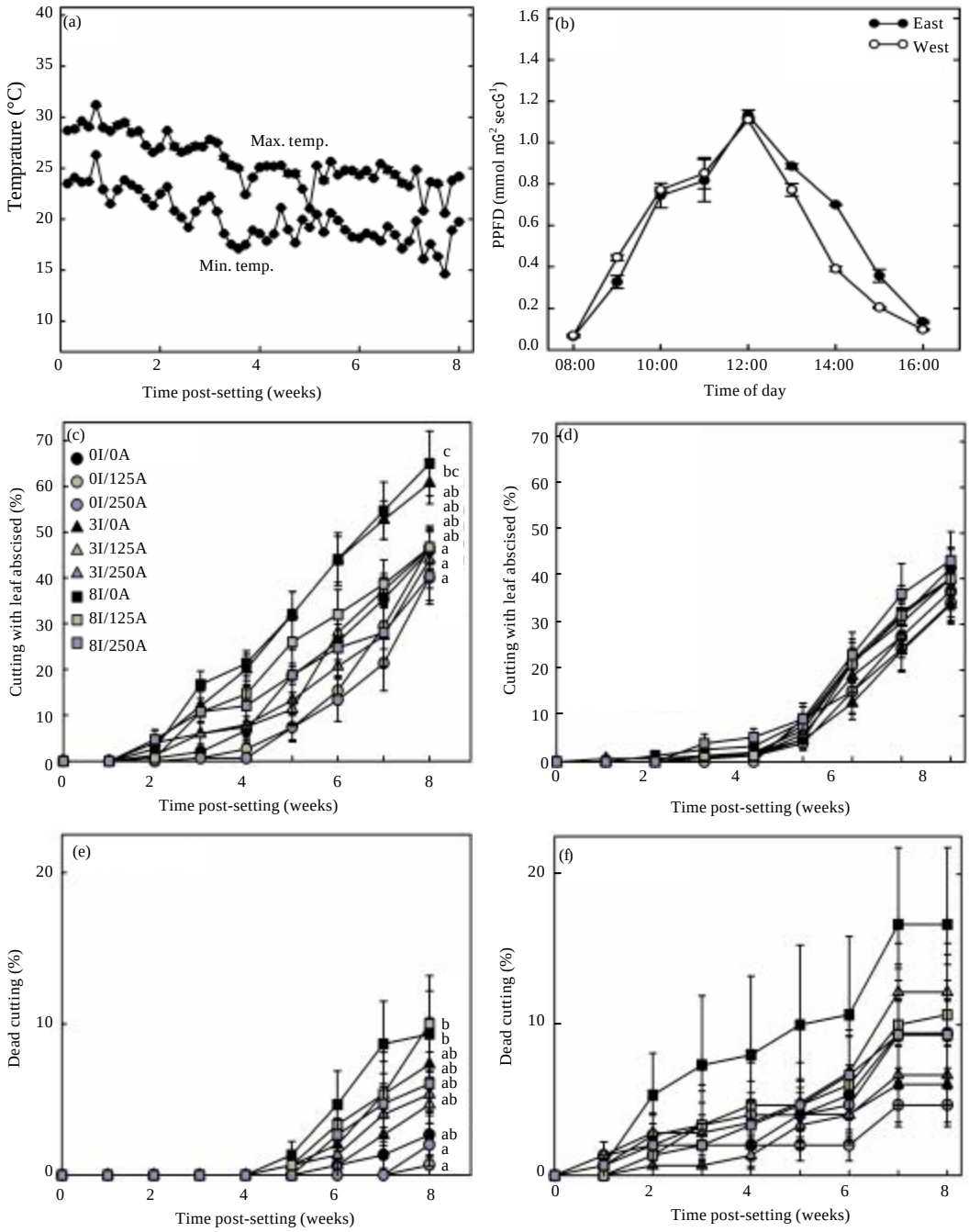

Fig. 3(a-f): Experiment 2: (a) Daily maximum and minimum temperatures and (b) Photosynthetic photon flux densities (PPFD) in the glasshouse, Percentage of cuttings with the leaf abscised of (c) *Corymbia torelliana*×*C. citriodora* Ct×Cc and (d) *Eucalyptus pellita*×*E. grandis* (Ep×Eg) and mortality rate of (e) Ct×Cc and (f)Ep×Eg cuttings subjected to different concentrations of IBA (I, g kg^{-1}) and AVG (A, mg L^{-1}), Final Mean±SE with different letters within a hybrid are significantly different (ANOVA and Duncan's multiple range test, $p<0.05$, n = 10)

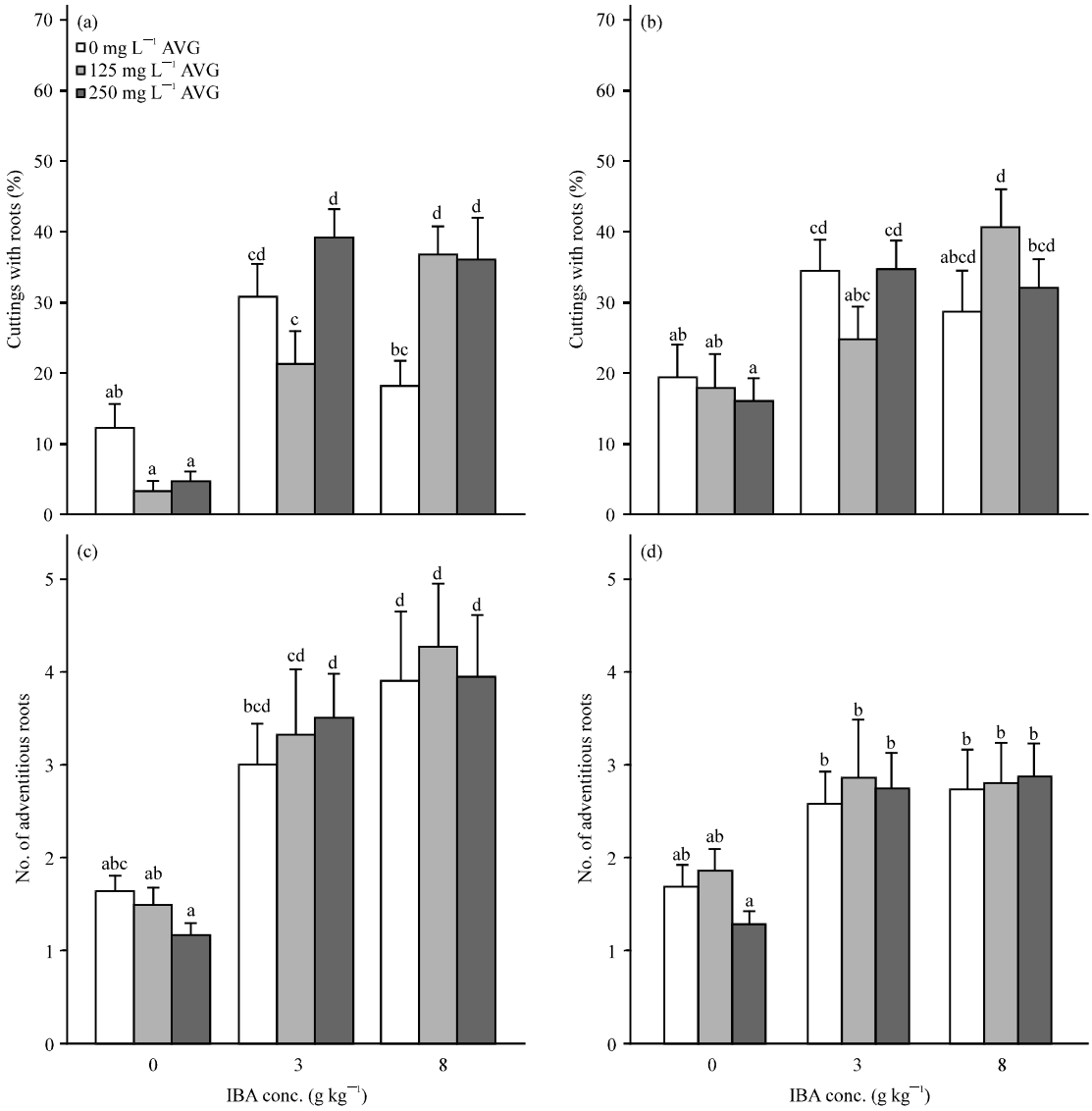

Fig. 4(a-d): Experiment 2: Percentage of cuttings with roots of (a) *Corymbia torelliana*×*C. citriodora* (Ct×Cc) and (b) *Eucalyptus pellita*×*E. grandis* (Ep×Eg) and Number of adventitious roots per rooted cutting of (c) Ct×Cc and (d) Ep×Eg cuttings subjected to different concentrations of IBA and AVG, Mean±SE with different letters within a hybrid are significantly different (ANOVA and Duncan's multiple range test, $p<0.05$, n = 4-10 for Ct×Cc and 7-10 for Ep×Eg)

treatments, 8 g kg^{-1} IBA with 0 or 125 mg L^{-1} AVG, resulted in higher mortality than for cuttings treated with 0 g kg^{-1} IBA and 125 or 250 mg L^{-1} AVG (Fig. 3e). For *E. pellita*×*E. grandis*, leaf abscission and cutting death did not vary significantly among the nine treatments (Fig. 3d, f). Abscission was generally greatest between 2 and 8 weeks after setting for *C. torelliana*×*C. citriodora* and between 4 and 8 weeks after setting for *E. pellita*×*E. grandis* (Fig. 3c, d).

More *C. torelliana*×*C. citriodora* cuttings formed roots when treated with 3 g kg^{-1} IBA with or without AVG, when compared with untreated cuttings (Fig. 4a). However, rooting was only

Table 1: Timing of adventitious root initiation and emergence in *Corymbia torelliana*×*C. citriodora* and *Eucalyptus pellita*×*E. grandis* cuttings

		Cuttings with root initiation (n = 5)		Cutting with root emergence (n = 5)	
Days post-setting	IBA (g kg^{-1})	*C. torelliana*×*C. citriodora*	*E. pellita*×*E. grandis*	*C. torelliana*×*C. citriodora*	*E. pellita*×*E. grandis*
7	0	0	0	0	0
7	8	0	1	0	1
14	0	2	1	1	0
14	8	1	0	1	0
21	0	0	1	0	1
21	8	0	1	0	1

increased using 8 g kg^{-1} IBA if the IBA application was followed by an application of 125 or 250 mg L^{-1} AVG (Fig. 4a). For *E. pellita*×*E. grandis*, three of the treatments, 3 g kg^{-1} IBA with 0 or 250 mg L^{-1} AVG and 8 g kg^{-1} IBA with 125 mg L^{-1} AVG, significantly increased the percentage of cuttings with roots compared with untreated cuttings (Fig. 4b).

The number of adventitious roots per rooted *C. torelliana*×*C. citriodora* cutting was increased following application of 8 g kg^{-1} IBA with or without AVG or following application of 3 g kg^{-1} IBA with 250 mg L^{-1} AVG (Fig. 4c). No treatment significantly affected the number of adventitious roots per rooted cutting, when compared with untreated cuttings, for *E. pellita*×*E. grandis* (Fig. 4d).

In *C. torelliana*×*C. citriodora*, mortality and adventitious root numbers of untreated cuttings were higher in Experiment 1 than Experiment 2 (Fig. 1e, 2c, 3e, 4c). In *E. pellita*×*E. grandis*, defoliation was lower and the percentage of cuttings with roots was higher in Experiment 1 than Experiment 2 (Fig. 1d, 2b, 3d, 4b). Other means for untreated cuttings did not differ significantly between experiments.

Timing of adventitious root formation: Microscopic adventitious roots were found within 14 days of setting for both *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis* (Table 1). Stems of both hybrids had a central pith region, surrounded by vascular tissue containing xylem, cambium and phloem arranged in a rectangular or, less commonly, a circular pattern. This was surrounded by cortical tissue with a single layer of epidermis cells (Fig. 5a, b). Adventitious root primordia arose at or near the cambium (Fig. 5c, d). Adventitious roots emerged through the epidermis by 14 days after setting in both *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis* (Table 1, Fig. 5e, f).

DISCUSSION

This study developed improved propagation techniques for the eucalypt hybrids, *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis*. Application of the auxin, IBA, with or without the ethylene inhibitors, MCP or AVG, frequently increased the percentage of cuttings that formed adventitious roots or the number of adventitious roots per rooted cutting. IBA sometimes increased defoliation and death of *C. torelliana*×*C. citriodora* cuttings, as found previously (Trueman and Richardson, 2008), but AVG alleviated these effects and also increased the percentage of cuttings that formed roots. There appears ample opportunity for MCP or AVG application after IBA application but prior to defoliation because root initiation had already occurred within 14 days of setting, well before the main phases of cutting defoliation and death.

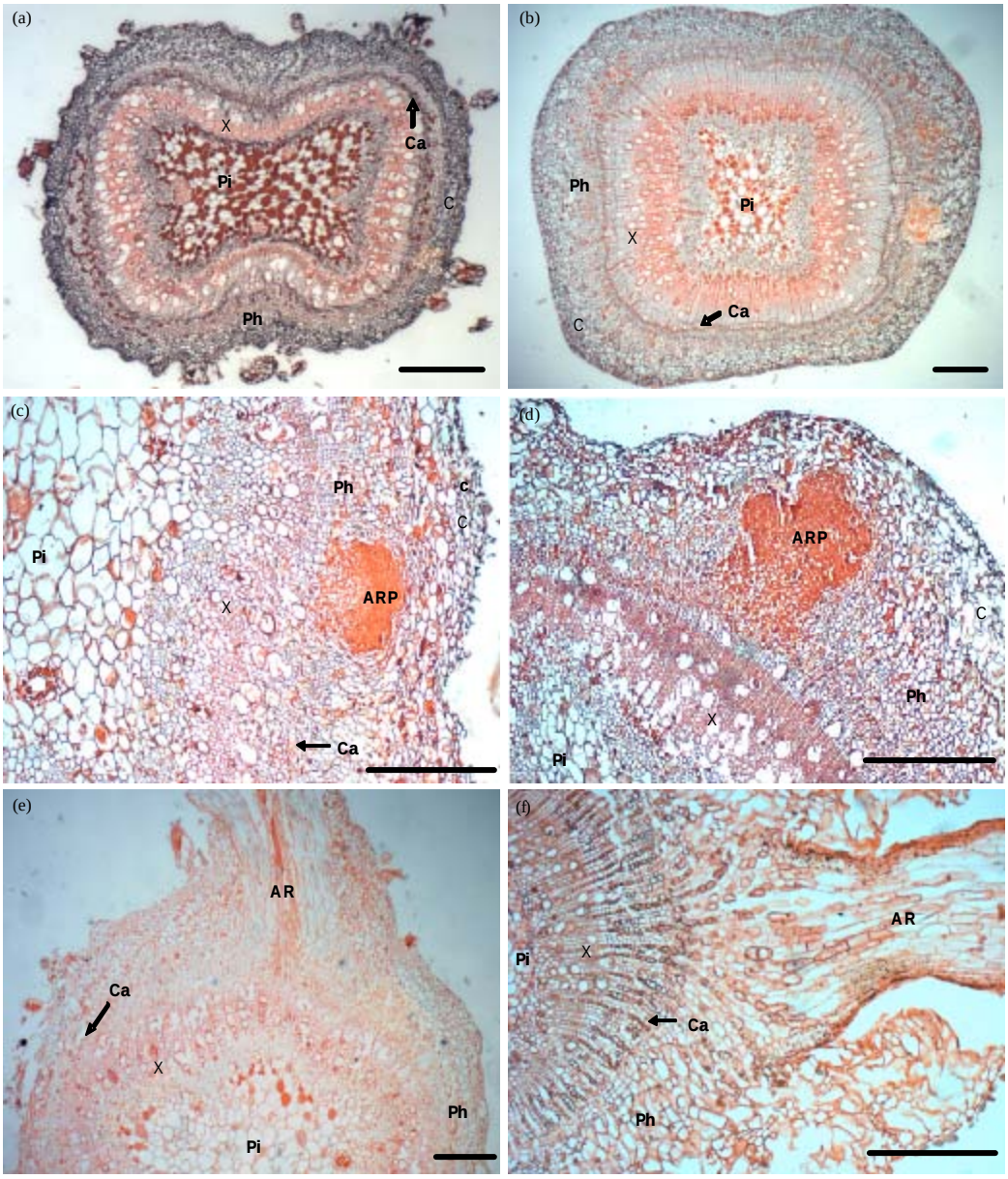

Fig. 5(a-f): Stem anatomy, adventitious root primordia (ARP) and root emergence at the base of eucalypt cuttings (transverse sections), (a) Stem of *Corymbia torelliana*×*C. citriodora* (Ct×Cc) at 21 days after setting (0 g kg^{-1} IBA), (b) Stem of *Eucalyptus pellita*×*E. grandis* (Ep×Eg) at 14 days after setting (0 g kg^{-1} IBA), (c) Adventitious root primordium in Ct×Cc at 14 days after setting (0 g kg^{-1} IBA), (d) Adventitious root primordium in Ep×Eg at 14 days after setting (0 g kg^{-1} IBA), (e) Adventitious root emerging from Ct×Cc at 14 days after setting (8 g kg^{-1} IBA) and (f) Adventitious root emerging from Ep×Eg at 21 days after setting (0 g kg^{-1} IBA). Scale bars: 150 μm, AR: Adventitious root, Ca: Cambium, C: Cortex, Pi: Pith, Ph: Phloem, X: Xylem

IBA application in the absence of MCP or AVG had promotive, although inconsistent, effects on the percentage of cuttings with roots and the number of adventitious roots per rooted cutting. Similar results were found previously with *C. torelliana*×*C. citriodora*, where 3 g kg^{-1} IBA increased the percentage of cuttings with roots and 8 g kg^{-1} IBA increased the number of adventitious roots per rooted cutting on some but not all, occasions (Trueman and Richardson, 2008). IBA increases the percentage of *C. torelliana*, *E. dunnii*, *E. grandis*, *E. grandis*×*E. urophylla*, *E. nitens* and *E. pellita*×*E. tereticornis* cuttings that forms roots (Wendling *et al*., 2000; Zuffellato-Ribas and Rodrigues, 2001; Luckman and Menary, 2002; Trueman and Richardson, 2008; Trueman *et al*., 2012). However, high IBA doses are less effective than intermediate doses in *C. torelliana*×*C. citriodora*, *E. grandis*×*E. urophylla* and *E. pellita*×*E. tereticornis* (Wendling *et al*., 2000; Trueman and Richardson, 2008) and high IBA doses can actually reduce rooting percentages in *C. citriodora*, *E. grandis* and *E. grandis*×*E. urophylla* (Wendling and Xavier, 2005; Goulart *et al*., 2008; Trueman and Richardson, 2008).

The highest dose of IBA (8 g kg^{-1}) increased defoliation and death of *C. torelliana*×*C. citriodora* cuttings in the experiment conducted under higher irradiance. Defoliation commenced in the second week after setting, similar to the timing (5-8 days after setting) observed on *C. citriodora* and *C. henryi* cuttings with the same IBA dose (Catesby and Walker, 1998). Defoliation has been observed previously at this dose for *C. citriodora*, *C. torelliana* and *C. torelliana*×*C. citriodora* cuttings (Trueman and Richardson, 2008) and defoliation is evident in tissue cultures of *C. citriodora* and *C. maculata* when shoots are induced to form roots by NAA or IBA, respectively (Nagae *et al*., 1996; Steinitz *et al*., 2010). IBA does not reduce chlorophyll fluorescence of *C. citriodora*, *C. torelliana* or *C. torelliana*×*C. citriodora* cuttings (Trueman and Richardson, 2008), indicating that IBA does not cause photosystem down-regulation or damage in their leaves. However, Steinitz *et al*. (2010) described increased root induction for *C. maculata* shoots *in vitro* after combining STS which inhibits ethylene action, with IBA. These results indicate that auxin-stimulation of ethylene production (Sun and Bassuk, 1993; Zhu *et al*., 2008) may be the primary cause of leaf abscission in eucalypt cuttings. The present findings support the hypothesis that auxin-stimulated defoliation of *Corymbia* cuttings is caused by ethylene production and that high ethylene production is detrimental to rooted cutting production.

An interesting feature of the current study was that the level of defoliation on untreated cuttings of both *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis* was not affected by MCP or AVG application. This suggests that the natural levels of leaf abscission in the propagation environment are not the direct result of high ethylene accumulation and that only the additional level of abscission induced by IBA is caused by ethylene. Indeed, MCP had no effect on defoliation during the first experiment, in which IBA did not induce defoliation. IBA-induced mortality has been found previously (Trueman and Richardson, 2008), as well as in the current study, to vary greatly from one setting to the next and it appears that ethylene inhibitors may only be effective in those settings where IBA increases defoliation. There have been few reports on the use of ethylene inhibitors for propagation of cuttings or *in vitro* shoots. STS promotes rooting of mung bean cuttings at a high NAA dose (De Klerk and Hanecakova, 2008) and induces rooting of IBA-treated *C. maculata* shoots (Steinitz *et al*., 2010). However, MCP, AVG and STS are used extensively to inhibit or delay ethylene-induced leaf senescence, flower senescence, fruit abscission and fruit ripening (Adkins *et al*., 2005; Zhu *et al*., 2008; Shimizu-Yumoto and Ichimura, 2010).

The timing of root initiation may be critical in determining the optimal application time for ethylene inhibitors, because some ethylene could be beneficial during the initiation phase of root formation (De Klerk and Hanecakova, 2008; Mendes *et al*., 2011). However, MCP and AVG had no effects on adventitious root formation in eucalypt cuttings that were not treated with IBA. Adventitious root primordia and root emergence were evident by 14 days after setting for both *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis* cuttings, very similar to the time of root emergence (15 days after setting) in cuttings of *E. globulus*×*E. maidenii* (Schwambach *et al*., 2008). Root induction and root initiation, therefore, preceded the main phases of defoliation and death which typically commenced 2-4 and 5-7 weeks after setting, respectively. The vascular tissue in stems of both eucalypt hybrids was arranged in a rectangular pattern and root primordia were observed in the phloem before roots emerged through the cortex and epidermis. Root initiation occurred at or near the vascular cambium, consistent with the sites in many other woody species (Hartmann *et al*., 1997).

CONCLUSION

This study has shown that treatments that combined an auxin with an ethylene inhibitor increased rooted cuttings production and improved root system quality. For instance, combining IBA (8 g kg^{-1}) with MCP (400 nl L^{-1}) or AVG (125 mg L^{-1}) increased the number of *C. torelliana*×*C. citriodora* rooted cuttings by 83 and 206%, respectively and increased the number of *E. pellita*×*E. grandis* rooted cuttings by 46 and 110%, respectively. On average, these *C. torelliana*×*C. citriodora* plants possessed 2.2 and 2.7 more adventitious roots and the *E. pellita*×*E. grandis* plants possessed 1.0 and 1.1 more adventitious roots, than untreated cuttings. These propagation techniques will allow deployment of *Corymbia* and *Eucalyptus* hybrids through a clonal forestry or vegetative family forestry program, whereby plantation trees are produced from large numbers of cuttings harvested from a limited supply of seedlings. However, research is required to optimise the propagation techniques because rooting percentages (e.g., 30-42 and 29-59% from optimal treatments for *C. torelliana*×*C. citriodora* and *E. pellita*×*E. grandis*, respectively) remain below the 70%-level preferred by commercial nurseries.

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