Characterising wood properties for deployment of elite subtropical and tropical hardwoods

Final Report

Stephen J. Trueman[‡*], Geoff R. Dickinson[‡*], John R. Huth[*], Anton Zbonak[*], Jeremy T. Brawner[†], Kevin J. Harding[*], David J. Lee[‡*], Paul Warburton[†], Tracey V. McMahon[‡], Amanda J. Kilkenny[‡], Laura Simmons[‡] and Helen M. Wallace[‡]

[‡]Faculty of Science, Health, Education & Engineering, University of Sunshine Coast

[*]Horticulture and Forestry Science
Agri-Science Queensland
Department Employment, Economic Development and Innovation

[†]CSIRO Plant Industry

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Introduction and Summary

Queensland has over 42,000 hectares of hardwood plantations, with 13,700 hectares currently managed for sawn timber and high-value products. Previously, a major impediment to expansion of the hardwood sawn timber and high-value products industry in Queensland was that improved varieties of the key subtropical and tropical species were not available for plantation establishment.

Trees from earlier projects, such as Hardwoods Queensland and the Private Plantations Initiative, have now reached an age where selection for growth, form and wood properties is possible. The current project used non-destructive and destructive wood evaluation techniques to characterise the timber quality of 443 subtropical and tropical *Corymbia* and *Eucalyptus* trees in these plantings, allowing selection of trees with the best growth, form and wood properties under Queensland conditions. Ecological assessments were also undertaken in the *Corymbia* plantings to identify germplasm that posed minimal risk of gene flow into native forests. Elite varieties are being fast tracked for deployment in Queensland using economical systems for germplasm capture and nursery production.

The project identified and captured 108 new *Corymbia* and *Eucalyptus* varieties that can be grown with confidence in Queensland over a shorter rotation length and which produce well-characterised high-quality hardwood timber.

The 60 *Corymbia* hybrid varieties selected in this project are being captured as cuttings for clonal field and propagation tests in conjunction with Clonal Solutions Australia Pty Ltd (CSA). These trials will be established in 2012–2014 as bulked-up varieties become available. CSA will provide DEEDI with ramets of the better rooting clones for testing on commercial growers' land. Ultimately, a subset of the clones that combine the attributes of elite growth, disease resistance, form, wood properties and propagation potential will be bulked up to provide the hardwood plantation sector with a new tranche of elite *Corymbia* hybrid clones that are more stringently tested than the eight commercial clones currently available. Commercial clones released as part of this study will have significantly increased growth, disease resistance, form, heartwood percentage and straightness, with increased performance in the order of 30% over that of plantations established using wild *Corymbia* species seed.

The 24 *Eucalyptus argophloia* and 24 *Eucalyptus cloeziana* varieties selected in this project are being captured as grafts for clonal seed orchards that will be established in 2012–2013 on industry partner land. Plantations established using seed from these seed orchards (expected to be available by 2015) will have increased performance in the order of 20% due to better growth, straightness and form, and increased heartwood percentage over plantations established using wild seed.

The *Eucalyptus pellita* trees in this project were severely damaged by Cyclone Yasi in January 2011, and so it was not feasible to capture elite trees based on growth, form and wood properties. If a plantation grower wishes to plant *Eucalyptus pellita* in Queensland's wet tropics, then new trials can be established rapidly using seed in stock. It will still be possible to benefit from the information captured by this project to develop *Eucalyptus pellita* trees with large proportions of heartwood, desirable colour and wood density.

All the elite varieties from this project will be screened for resistance to myrtle rust. The *Corymbia* hybrid clones will also be screened for *Quambalaria* resistance. This will be on-going work over the next few years as material becomes available.

This project also developed a preliminary risk assessment framework for gene flow from *Corymbia* hybrid plantations into native forests. This framework indicated a moderate to high risk of some pollen-mediated gene flow from *Corymbia* hybrid plantations into nearby native populations of *Corymbia citriodora*. There is also a risk that a very small proportion of *Corymbia* hybrids will possess the suite of characteristics that allow dispersal of their seeds into native forests by native bees. However, only about 1.5 trees in every 1000 will have these seed dispersal characteristics, which equates to approximately one tree per hectare. We discuss possible mitigation measures to reduce the potential risks of gene flow.

This report provides a comprehensive account of achievements in the three key project areas:

1. Wood analysis of tropical and subtropical *Corymbia* and *Eucalyptus* trees;
2. Optimal propagation systems for elite *Corymbia* and *Eucalyptus* germplasm; and
3. Potential gene flow risks from Corymbia plantations.

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Chapter 1. Wood Analysis of tropical and subtropical *Corymbia* and *Eucalyptus* trees

1.1 Sourcing of material for studies

There are four taxa in this project: *Eucalyptus argophloia*, *E. cloeziana*, and *E. pellita* and *Corymbia* hybrids[1]. This material has been sourced from trials across Queensland (Table 1.1). The locations of these trials are shown in Appendix 1 and a brief site description of each trial is shown in Appendix 2.

Table 1.1. Location of trials from which material was sourced for this project.

Taxa	Experiment No.	Location
E. argophloia	460a HWD	Dunmore State Forest Office
	460b HWD	'Glengarry' – DEEDI Dalby Agricultural College
	460e HWD	Dunmore State Forest Office
E. cloeziana	481d HWD	Cpt 56 St Marys LA SF 57 St Mary
E. pellita	767a ATH	Forestry Plantations Queensland (FPQ), Ingham nursery site
Corymbia hybrids	469d HWD	Amamoor – FPQ's Poulson's block
	394a HWD	Devils Mountain, Sexton – FPQ's Mulholland's block
	394b HWD	Mt McEuan, Hivesvile – FPQ's Stumer's block

1.2 Methodology for non-destructive evaluation (NDE) of wood properties

1.2.1 Standing tree acoustic wave velocity and Pilodyn penetration measures

Standing tree acoustic wave velocity was measured using a Fakopp Microsecond Timer (Plate 1.1). The instrument measures the time of flight between two pins spaced at a set distance (usually 1 m) apart. Sampling was done on the northern and eastern face of the stem when the tree was straight; when the tree was leaning the sample location was altered to 90 degrees to the lean and parallel with the lean. This measurement is used to predict wood stiffness.

An estimate of wood density was measured by a 6-Joule Pilodyn fitted with a 2.0 mm diameter pin (Plate 1.2). Bark windows, about 4 cm × 8 cm, were created to expose the wood. Pilodyn penetration measurements were made on both the eastern and western wood surfaces; unless the tree was leaning in this circumstance the sample location was altered to 90 degrees to the lean.

These two evaluations were undertaken on all taxa.

[1] A hybrid between *Corymbia torelliana* (female parent) and either *C. citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegata*, *C. henryi* or *C. maculata* as the male parent.

Plate 1.1. Using a Fakopp Microsecond Timer – Martin Davies and Terry Copley

Plate 1.2. Using a 6-Joule Pilodyn – Nick Kelly

Plate 1.3. Taking a 12 mm wood core – Paul Macdonell

1.2.2 Wood Coring

Bark-to-bark wood cores (12 mm diameter) were taken at approximately 1.2 m above ground level starting on either the eastern or western face of the tree, for correlation purposes these were oriented with the bark windows used for Pilodyn pin penetration readings (Plate 1.3). Bark was removed in the first drill thrust; the drill bit was cleaned before core drilling. Once the core was extracted, the core ends were marked with the east and west orientation. Cores were then placed in a plastic bag and stored in a car fridge prior to being sent to the Wood Quality Improvement Laboratory of DEEDI at Indooroopilly for determination of sapwood width, heartwood proportion and basic density of heartwood and sapwood segments.

Colour measurement was also assessed on core samples for *E. pellita.* Diametric core samples were cut radial along pith into two halves to expose radial-longitudinal face. Samples were air-dried and planned to obtain a smooth surface to highlight the colour variation. On each sample, several surface spots were identified representing true heartwood. A MiniScan XE portable colour measurement spectrophotometer from HunterLab was used to measure colour characteristics. The measurements of colour were performed for air-dried samples and after seven days placed under ultraviolet lamp to simulate sun exposure. The aperture size was 12 mm diameter. Colour is expressed according to the Commission International de l'Eclairage (CIE) *L*a*b** colour space (abbreviation CIELAB) with a standard illuminant *D*65 and a 10º standard observer. The *L** parameter represents lightness where the values of *L** vary from 0 (black) to 100 (white). The a* and *b** parameters describe the chromatic coordinates on green-red (*a**) and blue-yellow (*b**) axes.

1.3 Methodology for destructive evaluation of wood properties

The processing method for destructive evaluation of wood properties in the project has been switched from sawing that was initially proposed to peeling for logistical and practical reasons (following discussion with the DEEDI fund coordinator) and to build on collaborative efficiencies between the Smart Forest Alliance Queensland (SFAQ) and other Plantation Hardwoods Research Fund (PHRF) projects. As tree size in the trials under evaluation was small the resulting logs were also better suited to peeling than to sawing. Previous sawing studies conducted under other projects for similar species have provided encouraging results. However, veneering provides an important potential value-adding option for young / small diameter logs that cannot be sawn. This destructive sampling made it possible to calibrate and validate the non-destructive sampling ensuring the trees within a species for each trait could be ranked.

The results obtained from the non-destructive sampling were used to select trees screened in the destructive sampling. Trees destructively harvested were selected from the full range of measured wood properties for each trait and from a range of provenances and families to account for variation in the taxon. Selected trees were relatively straight without defects (no sweep and no large branches). One 1.5 m long billet was taken from the butt section from each selected tree. In addition a 20 mm wide disk was taken from the top end of the billet for calculation of basic density and the proportion of heartwood and sapwood.

The 1.5 m billets were peeled using an 'Omeco' spindleless lathe (Plate 1.4) at the Salisbury Research Centre (DEEDI) to produce veneer ribbon with target thickness of 2.8–3.0 mm.

Veneer ribbon (Plate 1.5) was trimmed into smaller manageable sheets and air-dried. Processing of two species (*Corymbia* hybrids and *E. pellita*) overlapped with another PHRF project – 'High value timber composite panels from hardwood plantation thinnings'. The processing results (net recoveries, veneer grade distributions) are the subject of a separate report due October 2012.

Plate 1.4. Processing a peeling billet in the 'Omeco' spindleless lathe – Eric Littee

Plate 1.5. A sample of veneer sheet – Fred Lane

Acoustic properties of the dried peeled veneer were conducted using the BING[2] method on veneer sample at three assessment points: close to peeler core (representing the inner part of the billet); in the middle of the veneer; and at opposing end of ribbon representing the outer part of billet.

E. argophloia and *Corymbia* hybrids had an additional 600 mm long billet taken above the first 1.5 m billet for stiffness determination on a small clear section using the 'Shimadzu' testing machine. It was used to test static bending strength (stiffness) and provided the modulus of elasticity (MOE[3]) and modulus of rupture (MOR[4]) for each sample. Only these two taxa were tested using the Shimadzu testing machine (this was an additional evaluation method not planned in the original project proposal), resulting in more information on these taxon. This additional work also made it possible to establish a correlation with Fakopp standing tree acoustic wave velocity and wood stiffness for these taxa.

There have been no previous studies undertaken to link standing tree NDEs to veneering results in subtropical and tropical plantation eucalypts in Australia. Project results examined how consistent the ranking of elite trees using non-destructive standing tree assessments compared to traits assessed during processing. For example, the project established the degree of correlation between acoustic wave velocity (Fakopp) versus veneer MoE, and density (Pilodyn penetration versus basic density).

Although not a requirement of this project, an opportunity was taken to gather stem taper measurements for volume equations compilation on trees of *E. argophloia* (five trees) and *Corymbia* hybrids (16 trees) – results not presented.

1.4 Results

1.4.1 Eucalyptus argophloia

Sixty-seven trees of *E. argophloia* from three experiments (Expt 460a HWD– 29 trees and Expt 460e HWD– 38 trees at Dunmore and Expt 460b HWD– 14 trees near Dalby) were selected for non-destructive testing. Trees were selected for non-destructive testing following a genetic analysis of all available growth and form data from these trials. Individual tree breeding values were predicted for height, diameter and form traits using a multivariate model that weighted data from

[2] BING is a method of measuring dynamic Modulus of Elasticity (MoE) through the use of acoustic resonance developed by Dr Henri Bailleres and his team at CIRAD. This method uses frequency as opposed to the time of flight used by other systems.
[3] MoE – modulus elasticity, is defined as the material's tendency to be deformed elastically (i.e., non-permanently) when a force is applied to it. A stiffer material will have a higher elastic modulus.
[4] MoR – modulus of rupture defined as a material's ability to resist deformation under load.

each site according to accuracy of predictions. Selection indices were then generated to provide a composite trait for ranking and subsequent short listing of trees; these trees were then verified by visual inspection in the field before the trees were confirmed as selections. The selected trees covered three sub-provenances (Burncluith, Burra Burri and Fairyland) of the species, effectively covering the available genetic variation of the species (Table 1.2). Diameter at breast height (DBH) and height was measured at age 12.7 years in December 2009.

1.4.1.1 Non-destructive evaluation

Non-destructive testing and tree coring were carried out in April 2010 at age 13.5 years.

Table 1.3 provides the average results at family level for all measured tree and wood properties. On average the heartwood ratio was 48% (range 16–66 %) and the weighted[5] basic density for the whole core was 729 kg/m^3 (range 648–806 kg/m^3).

There was a strong negative correlation between outer wood core (sapwood) basic density and Pilodyn pin penetration explaining about 66% of the variation (Figure 1.1). The results suggest that the Pilodyn tool could be used to rank families for basic density.

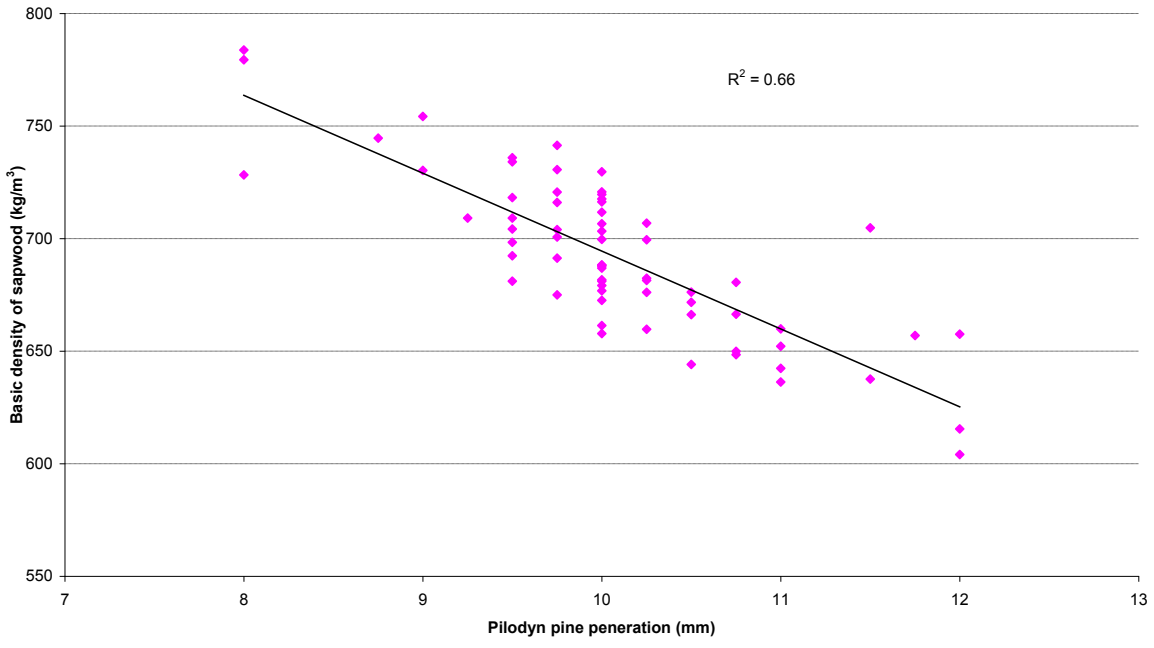

Figure 1.1. Relationship between basic density of sapwood and Pilodyn pin penetration for *E. argophloia*.

[5] Weighted values for basic density were calculated to make provision for the proportional representation of the smaller area of wood from the inner part of disc and the larger area of wood from the outer part closer to the bark-end.

Figure 1.2 shows the variation between families for heartwood proportion; Figure 1.3 shows the radial distribution of basic density. The radial distribution of basic density for each provenance is shown in Table 1.3. Contradictory to other species in this report, wood density of *E. argophloia* decreased from inner wood to outer wood. This could be due to high amount of extractives content deposited in the heartwood of this species.

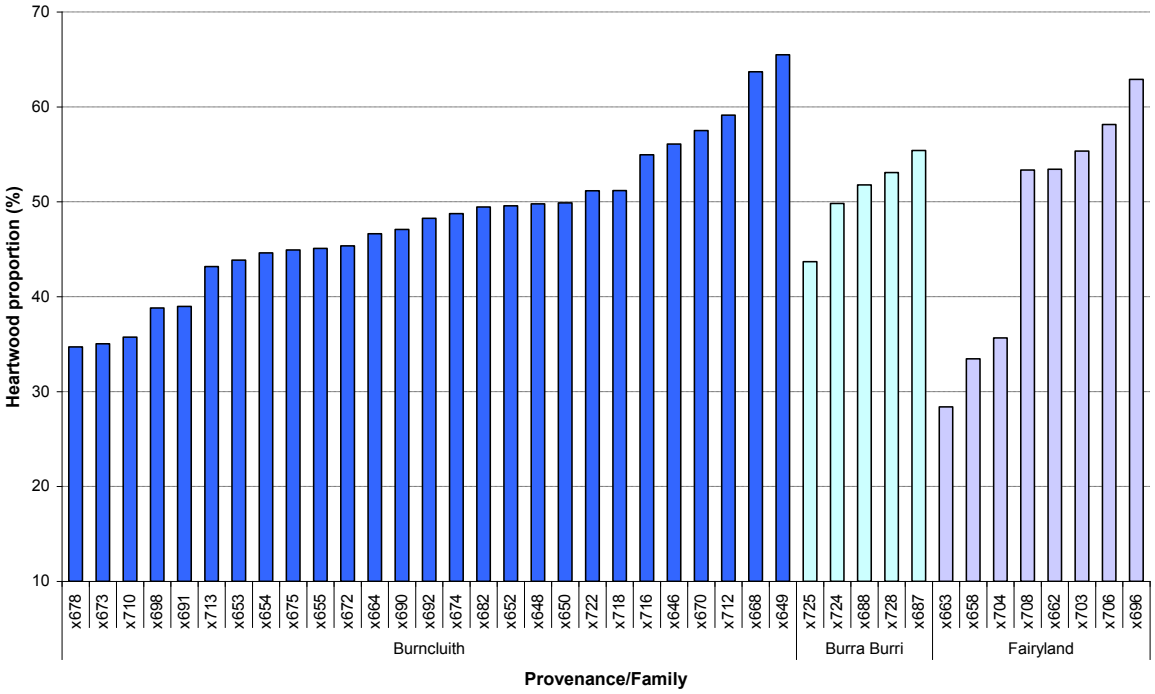

Figure 1.2. Variation of heartwood proportion (%) at age 13.5 years in *E. argophloia* families.

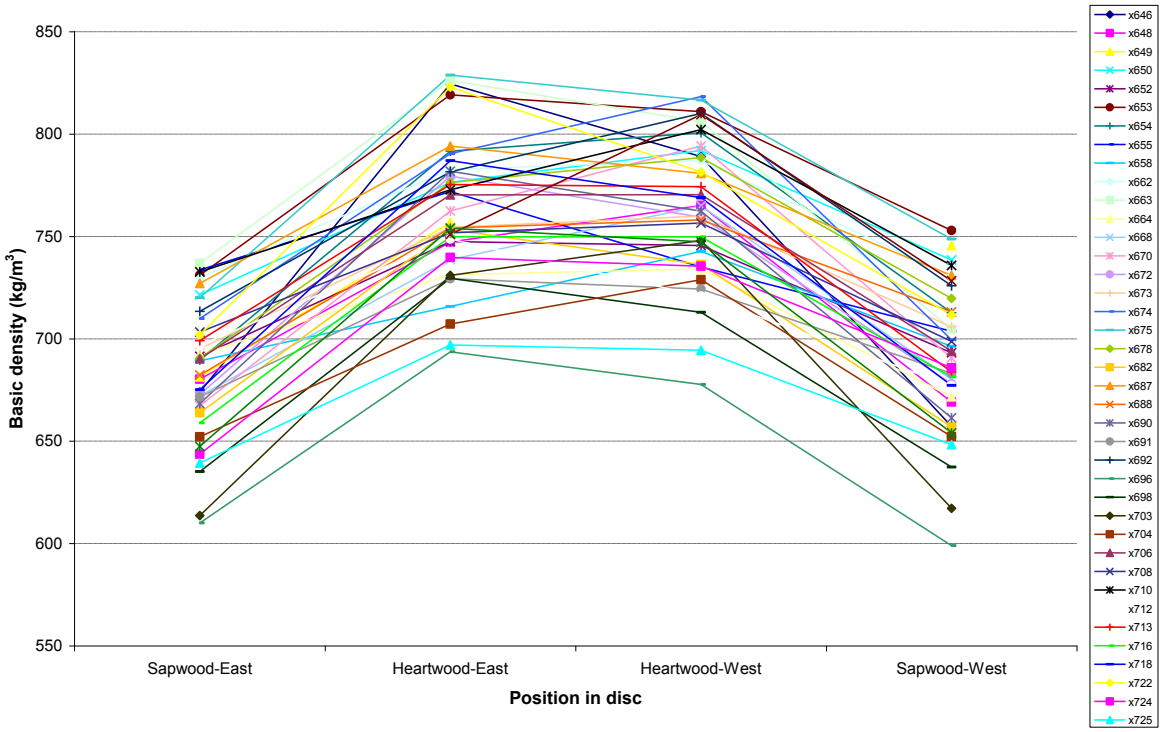

Figure 1.3. Radial distribution of basic density (kg/m^3) of 13.5-year-old *E. argophloia* samples in 39 families.

Table 1.2. Family average values of 67 selected trees measured tree growth and non-destructive wood properties.

Provenance	Family	DBH (cm)	Heart wood ratio (%)	Sapwood width (mm)	Pilodyn pin depth (mm)	Acoustic wave velocity (km/sec)	Basic density (kg/m^3)		
							Heartwood	Sapwood	Whole core*
Burncluith	x646	13.4	56.1	13.3	10.8	3.7	805	666	744
	x648	13.2	49.8	15.0	9.8	3.9	756	675	715
	x649	19.8	65.5	16.4	10.0	3.9	798	712	768
	x650	14.5	49.9	18.2	9.1	4.1	783	727	758
	x652	13.0	49.6	15.9	10.0	3.9	747	692	720
	x653	15.5	43.9	20.7	8.9	4.1	816	742	775
	x654	16.3	44.6	23.3	10.0	3.7	796	700	743
	x655	16.2	45.1	21.7	9.5	4.0	754	718	734
	x664	14.4	46.6	18.3	10.3	4.0	733	681	705
	x668	25.0	63.7	21.5	10.5	4.2	752	676	725
	x670	16.4	57.5	18.0	10.3	3.8	777	680	736
	x672	15.2	45.3	21.0	10.4	3.9	769	676	719
	x673	14.1	35.0	25.2	9.9	3.9	756	701	720
	x674	17.5	48.7	22.3	11.5	3.9	802	705	752
	x675	14.5	44.9	18.7	9.4	4.1	823	735	774
	x678	17.9	34.7	34.1	9.8	4.1	782	705	732
	x682	13.2	49.4	16.8	10.0	3.8	746	660	703
	x690	15.2	47.1	19.2	11.1	3.7	782	664	720
	x691	13.7	39.0	21.8	10.0	3.9	727	677	696
	x692	17.7	48.3	21.7	10.0	4.2	794	720	755
	x698	15.7	38.8	25.4	11.0	3.8	721	636	669
	x710	14.7	35.7	22.8	9.5	4.1	786	734	753
	x712	18.0	59.1	18.1	10.0	4.1	735	688	716
	x713	15.1	43.2	22.3	9.9	4.0	774	691	728
	x716	15.1	55.0	17.0	11.4	4.1	749	669	713
	x718	22.2	51.2	26.8	10.3	3.8	778	676	729
	x722	16.0	51.2	19.5	10.3	4.1	803	707	756
Provenance average		**15.5**	**47.4**	**20.4**	**10.1**	**4.0**	**772**	**695**	**732**
Burra Burri	x687	18.0	55.4	20.4	9.6	4.1	787	728	762
	x724	19.0	51.8	21.4	10.0	4.0	757	698	727
	x688	19.8	49.8	25.0	10.5	3.9	737	666	702
	x728	15.8	43.7	23.5	11.1	4.0	696	644	668
	x687	16.3	53.1	18.8	11.0	4.0	751	651	704
Provenance average		**17.6**	**51.7**	**21.3**	**10.3**	**4.0**	**754**	**688**	**722**
Fairyland	x658	14.7	33.5	27.5	9.8	4.1	727	693	705
	x662	13.5	53.4	16.6	9.0	4.2	785	730	759
	x663	13.7	28.4	27.3	9.8	3.8	818	721	748
	x696	20.5	62.9	18.5	12.0	3.9	686	604	655
	x703	17.8	55.3	20.0	12.0	3.9	739	615	684
	x704	13.9	35.7	25.4	11.0	4.1	717	652	675
	x706	19.0	58.1	18.0	9.8	3.9	770	693	738
	x708	20.8	53.3	24.0	9.5	4.0	769	701	738
Provenance average		**16.9**	**46.6**	**22.9**	**10.1**	**4.0**	**750**	**682**	**716**

* – weighted basic density

Table 1.3. Radial distribution of basic density (kg/m^3) for each provenance.

Provenance	Basic density (kg/m^3)				
	East-outer	East-inner	West-inner	West-outer	Average
Burncluith	691	771	770	699	732
Burra Burri	681	756	756	697	722
Fairyland	683	744	752	682	716
Average	688	764	764	695	727

1.4.1.2 Destructive evaluation

A sub-sample of five trees (four from Burncluith provenance and one from Burra Burri provenance) were selected from the Dunmore trials (Plate 1.6) with the destructive sampling undertaken during December 2010 at age 14 years. The results of wood properties from those five trees are presented in Table 1.4.

Standing tree acoustic wave velocity was a strong predictor of static MoE of clear samples with a R^2 of 98% (Figure 1.4).

Table 1.4. Wood property results from five destructively sampled trees – *E. argophloia.*

Property	Mean	Standard deviation	Minimum	Maximum
Static MoE – clear block samples (MPa)	14432	1263	13200	15837
Static MoR – clear block samples (MPa)	150	16	130	169
Sapwood width (mm)	24.1	2.4	20.3	26.1
Heartwood proportion (%)	44	4	39	48
Basic density – heartwood (kg/m^3)	773	10	757	783
Basic density – sapwood (kg/m^3)	488	80	405	612
Weighted basic density – whole disc (kg/m^3)	733	16	713	754
Radial unit shrinkage (12% to 5% moisture content)	0.28	0.02	0.26	0.32
Tangential unit shrinkage (12% to 5% moisture content)	0.39	0.02	0.37	0.41
Longitudinal unit shrinkage (12% to 5% moisture content)	0.009	0.004	0.002	0.012

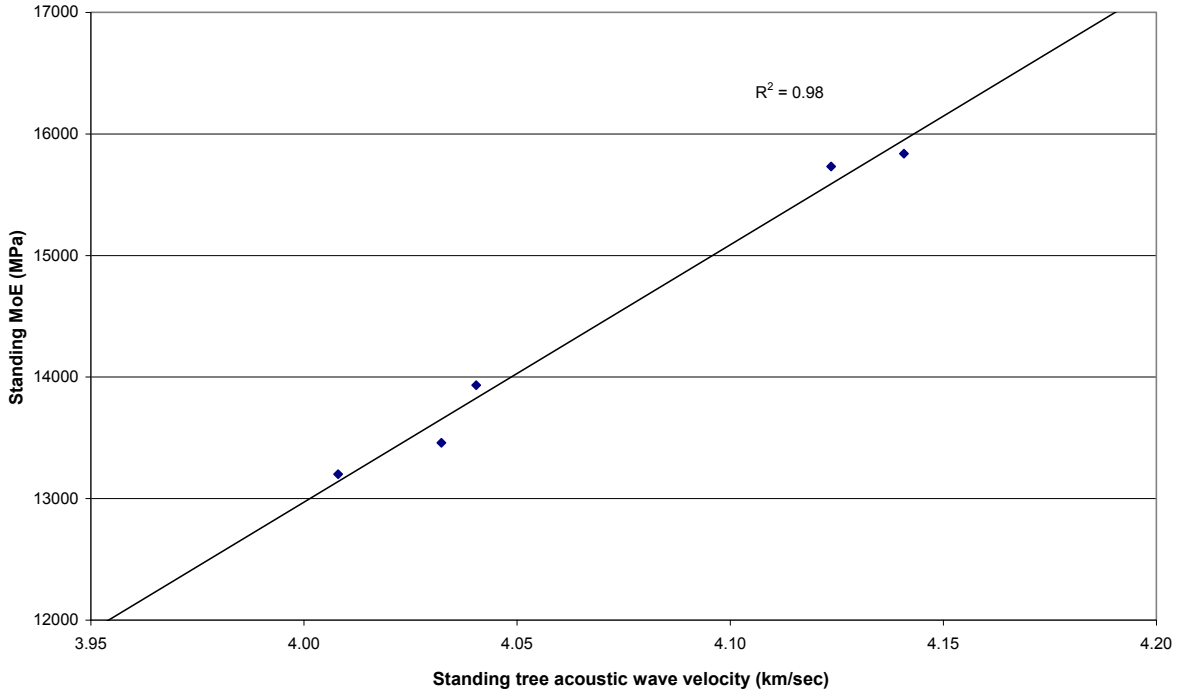

Figure 1.4. Relationship between static MoE measured on small clear samples and acoustic wave velocity of five *E. argophloia* trees.

The results of veneer stiffness assessed at three radial positions along the veneer length using the BING acoustic tool are provided in Table 1.5. There are also coefficient of determinations (R^2) of this measure and those from the standing tree acoustics using the Fakopp. Due to very narrow range in acoustic wave velocities detected by the Fakopp the coefficients of determinations were low and unable to predict veneer stiffness. This species was the only one that failed to have a correlation between the predicted stiffness of veneer samples using BING and the standing tree (Fakopp) predicted stiffness. This may be due to the lower basic density of the sapwood relative to that of the heartwood. This may be due to build-up of extractives in the heartwood. Further studies are required to clarify this.

Plate 1.6. *E. argophloia*
Plus tree 1ea2-048

Table 1.5. Veneer predicted stiffness (using the BING acoustic tool) at three different positions along the veneer length; coefficient of determinations between veneer stiffness and standing tree acoustic wave velocity using the Fakopp.

Parameter	Veneer positions			
	Inner core	Middle	Outer	Average
MoE (MPa)	7901	13856	15085	12281
R^2 with standing tree acoustic wave velocity	0.03	0.01	0.25	0.04

1.4.1.3 Selection of superior E. argophloia trees for grafting

Based on the wood property results 24 plus trees (Table 1.6) were selected for grafting; one tree is shown in Plate 1.6. These trees were from the three sub-provenances – Burncluith (13 trees), Burra Burri (five trees) and Fairyland (six trees). Coincidentally, 15 of the 24 selections identified in this study were trees that had been previously selected for growth and form based on earlier measures. Heartwood percentage was a key trait used in selection of these trees as most trees were above minimum thresholds for the other traits.

Table 1.6. A summary of the *E. argophloia* trees selected for grafting. A tick indicates the particular trait or wood property that was good in that tree.

Plus tree no.*†	Xname	Provenance	DBH	Basic density heartwood	Basic density sapwood	Per cent heartwood†	Straightness
1ea2-003	x712	Burncluith				✓	
1ea2-010	x653	Burncluith		✓	✓		
1ea2-012	x678	Burncluith				✓	
1ea2-033	x649	Burncluith		✓		✓	
1ea2-045	x654	Burncluith		✓		✓	
1ea2-046	x722	Burncluith		✓		✓	
1ea2-036	x668	Burncluith	✓			✓	
1ea2-038	x718	Burncluith	✓			✓	
1ea2-018	x670	Burncluith				✓	
1ea2-019	x650	Burncluith				✓	
1ea2-049	x692	Burncluith				✓	
1ea2-051	x673	Burncluith			✓		✓
1ea2-052	x650	Burncluith		✓	✓		
1ea2-014	x688	Burra Burri	✓			✓	
1ea2-044	x725	Burra Burri				✓	
1ea2-047	x726	Burra Burri	✓	✓	✓	‡	
1ea2-013	x687	Burra Burri	✓			✓	
1ea2-050	x687	Burra Burri				✓	
1ea2-007	x658	Fairyland				✓	
1ea2-043	x708	Fairyland				✓	
1ea2-004	x696	Fairyland	✓			✓	
1ea2-006	x708	Fairyland	✓			✓	
1ea2-048	x703	Fairyland				✓	✓
1ea2-035	x706	Fairyland	✓			✓	

* – Selections less than number 1ea2-044 were old selections
† – A ✓ indicates a heartwood proportion >41%
‡ – The sampling core for this tree was damaged so it was not possible to calculate heartwood proportion

1.4.1.4 Implications for future E. argophloia plantations

The trees selected in this study are being captured as grafts for a clonal seed orchard which will be established in 2012–2013 financial year on industry partner land. Plantations established using seed from this new clonal seed orchard (expected to be available by 2015) will have increased performance (in the order of 20%), due to better growth, straightness and form, and increased heartwood percentage over plantations established using wild seed. The information from this study will facilitate development of economic models of the potential growth and value of the timber of this species. This work is on-going.

1.4.2 Eucalyptus cloeziana

Seventy-two trees from Exp 481d HWD were selected for non-destructive testing. Trees were selected following a genetic analysis that combined all available growth and form data (DBH, height and straightness at four years and DBH at eight years,) into one multivariate analysis. A selection index combining growth and form (tree volume weight = 60% and tree form = 40%) was used to first identify superior families and subsequently identify the best trees within each family. Any trees with defects noted in the assessments were avoided and a field inspection was then undertaken to verify the selection process and select trees for subsequent wood quality sampling. Trees were selected from 24 families within 12 'provenances' from the two southern ecotypes of this species (Table 1.7). Diameter and height was measured in July 2009 at age 7.4 years

1.4.2.1 Non-destructive evaluation

Non-destructive testing and tree coring were carried out in July-August 2010 at age 8.4 years

Table 1.8 shows results of tree growth, standing tree and wood properties of core samples across selected families. On average the heartwood ratio was 55% (range 39–68%) and the weighted basic density for whole core was 594 kg/m^3 (range 503–661 kg/m^3).

Similarly as for *E. argophloia* there was a strong negative correlation between outer wood core basic density and Pilodyn pin penetration explaining about 66% of the variation (Figure 1.5). The results suggest that the Pilodyn tool can be used to rank *E. cloeziana* families for basic density. Figure 1.6 shows the radial distribution of basic density.

Table 1.7. Provenances for tree selection for non-destructive testing.

Ecotype	Provenance	No. families
Southern – coastal	2nd Generation*	2
	Como	2
	Goomboorian	1
	Home	4
	Neerdie	2
	SAFCOL†	1
	Toolara	2
	Veteran	3
	Wolvi	3
	Woondum	2
	Yurol	1
Inland	Cannidah	1
Total		24

* Gympie provenance
† Second generation bulk seed orchard seed from the South African Forest Company Ltd.

Table 1.8. Tree growth, standing tree and core wood properties for family averages – *E. cloeziana.*

Family	Ecotype	Provenance	DBH (cm)	Pilodyn pin depth (mm)	Acoustic wave velocity (km/sec)	Heartwood ratio (%)	Sapwood width (mm)	Basic density (kg/m^3)		
								Heartwood	Sapwood	Whole core *
x1420	Southern coastal	Como	22.3	11.3	4.0	60.9	19.5	575	626	595
x1427		Como	21.9	11.4	4.3	48.1	27.3	588	642	616
x4124		Goomboorian	23.0	11.4	4.3	58.8	21.4	559	636	591
x1390		Home	21.1	12.3	4.1	54.3	23.2	549	582	564
x4204		Home	20.9	11.7	4.2	51.0	24.8	523	612	568
x4209		Home	23.7	11.3	4.2	55.4	24.4	569	627	594
x4219		Home	25.1	11.8	4.1	52.5	27.8	565	626	594
x1416		Neerdie	22.6	12.8	4.1	54.2	23.9	525	599	559
x4147		Neerdie	22.6	10.1	4.1	54.1	24.7	592	667	626
x4196		Toolara	23.3	12.2	4.0	56.6	23.5	538	602	566
x4202		Toolara	22.4	11.3	4.3	56.9	22.8	604	672	633
x1399		Veteran	21.7	11.0	4.2	51.1	24.8	539	632	585
x4221		Veteran	24.3	10.5	4.1	64.2	19.6	597	663	621
x4222		Veteran	22.8	12.4	4.2	47.5	30.1	522	594	561
x1430		Wolvi	22.8	11.5	4.2	56.4	23.4	550	617	580
x1433		Wolvi	23.6	12.3	4.0	55.5	24.7	542	595	565
x4184		Wolvi	22.3	11.7	4.0	57.8	22.3	542	600	567
x1404		Woondum	21.6	11.7	4.1	56.6	21.3	565	625	591
x1406		Woondum	20.4	10.2	4.3	53.7	22.8	583	707	640
x1449		Yurol	23.2	11.8	4.2	58.3	21.8	550	628	582
Southern coastal ecotype mean			**22.6**	**11.5**	**4.1**	**55.2**	**23.7**	**559**	**628**	**590**
x4109	**Inland**	**Cannidah**	**21.6**	**10.2**	**4.3**	**57.9**	**19.6**	**603**	**677**	**634**
x4086	Southern coastal	2nd Gen	21.2	11.5	4.2	51.4	24.0	580	653	616
x4087		2nd Gen	21.1	10.8	4.3	56.2	21.6	574	666	614
2nd generation mean			**21.2**	**11.2**	**4.2**	**53.8**	**22.8**	**577**	**659.5**	**615**
x4121	Southern coastal	SAFCOL†	26.0	11.7	4.2	51.1	29.9	567	644	604
Overall mean			**22.6**	**11.5**	**4.2**	**55.1**	**23.7**	**562**	**633**	**594**

* Note weighted basic density
† Bulk seed orchard seed from the South African Forest Company Ltd.

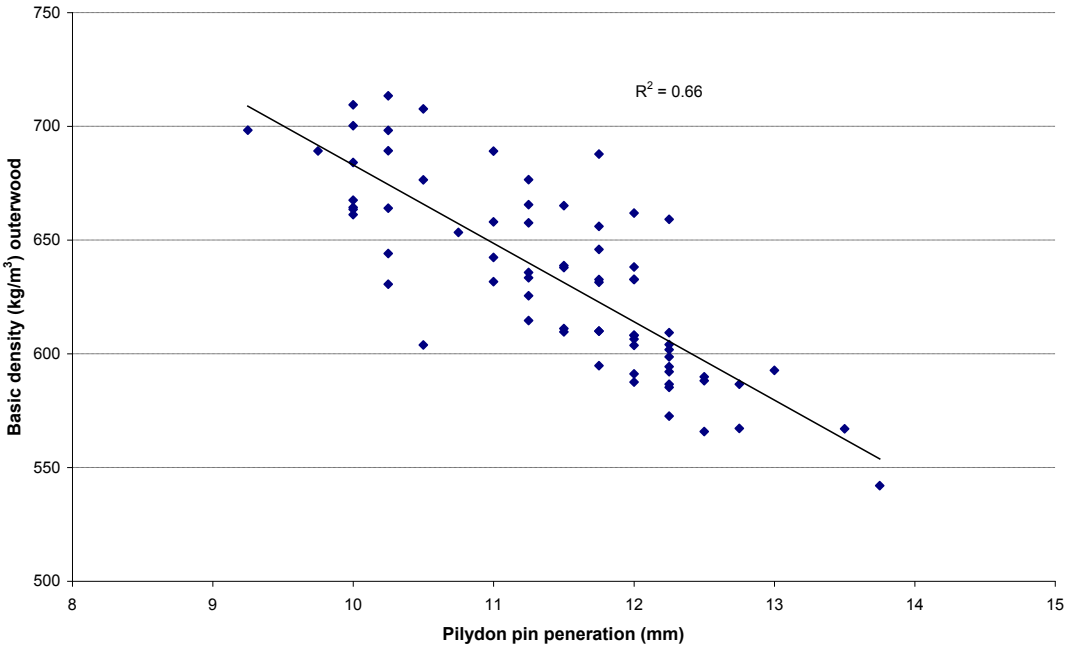

Figure 1.5. Relationship between basic density of outer wood and Pilodyn pin penetration for *E. cloeziana*.

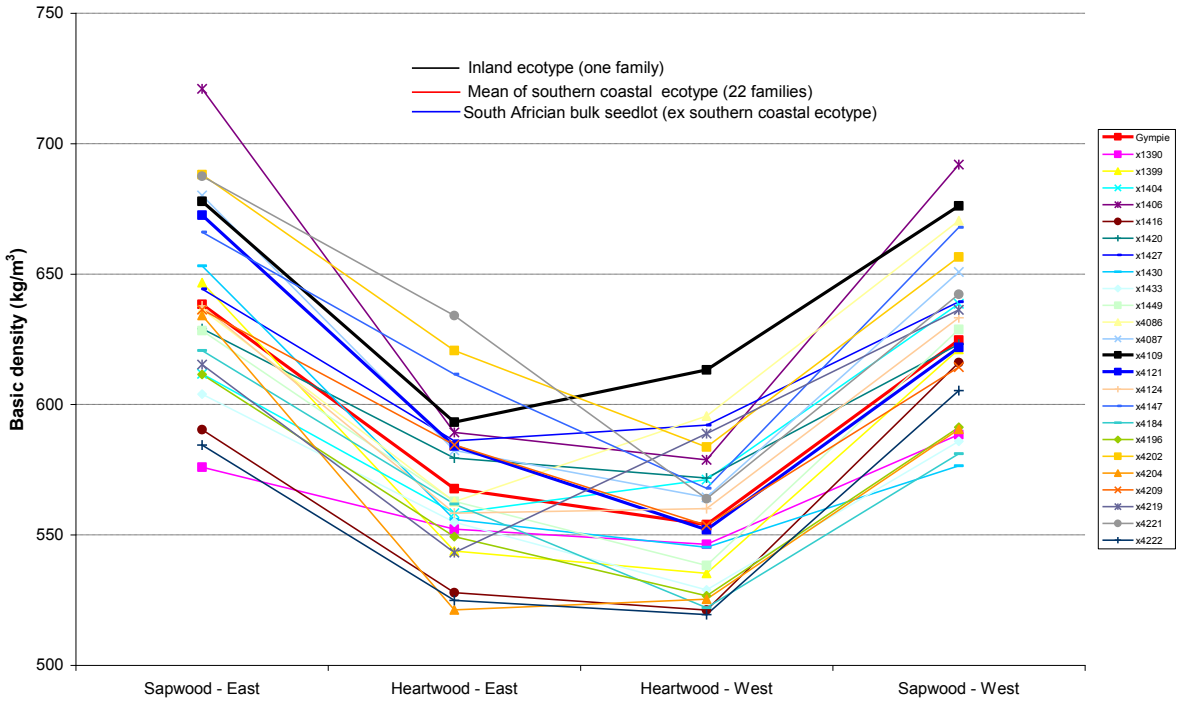

Figure 1.6. Radial distribution of basic (kg/m^3) density of eight-year-old *E. cloeziana* samples in 21 families from the southern coastal ecotype, one family from an inland ecotype and a bulk seedlot (x4121) from South Africa.

1.4.2.2 Destructive evaluation

Ten *E. cloeziana* trees (all first generation trees, one tree from each provenance except Cannidah and Como) were felled in July 2011 at age 9.4 years for destructive sampling. Prior to peeling the billets (Plate 1.7) were assessed for their stiffness (MoE) using the BING device (Plate 1.8).

Averaged results of wood properties of sampled trees are presented in Table 1.9. Standing tree acoustic wave velocity was strongly correlated with billet stiffness measured using the BING method explaining 82% of variation (Figure 1.7).

Table 1.9. Wood property results from 10 destructively sampled trees – *E. cloeziana*.

Parameter	Mean	Standard deviation	Minimum	Maximum
MoE (MPa) – using BING on billet	13036	2140	9816	16809
Sapwood width (mm)	24.8	2.7	20.6	27.9
Heartwood proportion (%)	53.8	4.1	46.7	57.9
Basic density – heartwood (kg/m^3)	545	36	507	622
Basic density – sapwood (kg/m^3)	619	45	567	707
Weighted basic density – whole disc (kg/m^3)	579	38	538	658

The results of veneer stiffness assessed at three radial positions along the veneer length for *E. cloeziana* are provided in Table 1.10 and also illustrated in Figure 1.8. Stiffness increased towards the outer part of the stem. Coefficient of determinations (R^2) between veneer stiffness and standing tree acoustic wave velocity (measured using the Fakopp tool) increased with an increase in distance from the inner core. This is logical as Fakopp stiffness is assessing the outer layers of the standing tree which are positioned closer to the veneer from the outer part of the tree. The R^2 of 0.76 between acoustic wave velocity and stiffness of outer veneer suggests that ranking for standing tree acoustic wave velocity assessment could be consistent with ranking based on actual veneer stiffness assessed during processing which would be beneficial for selection of elite trees for inclusion in both veneers and other solid wood products.

Plate 1.7. *E. cloe*ziana billets

Plate 1.8. Using BING

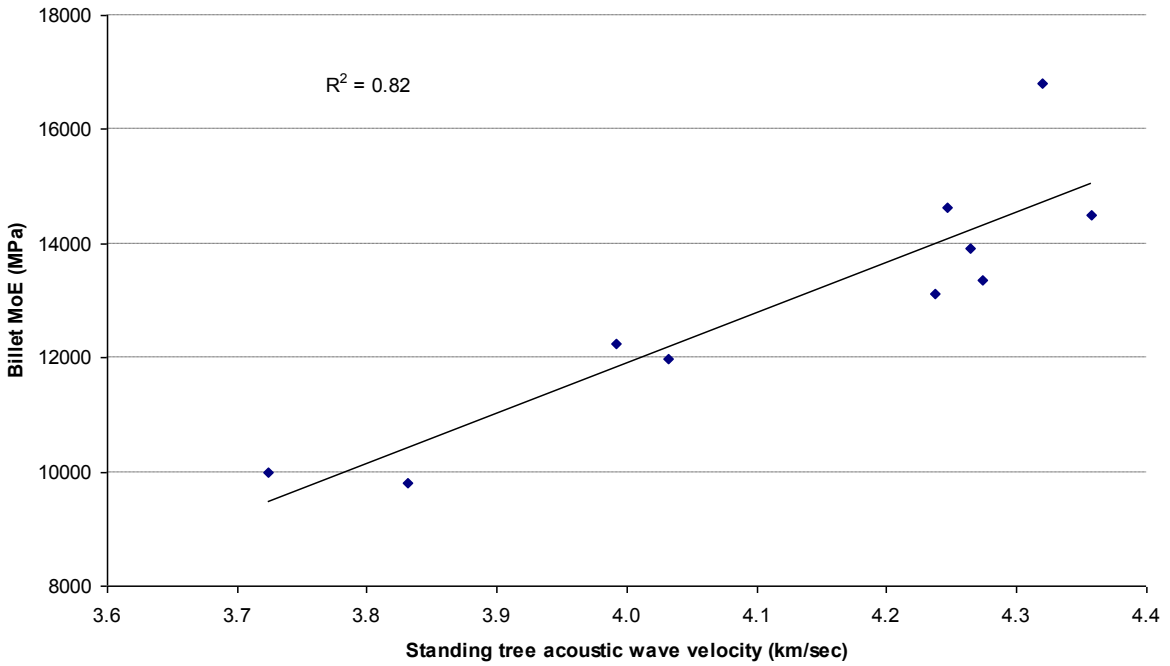

Figure 1.7. Relationship between predicted MOE for billets (from BING) and standing tree acoustic wave velocity (measured by Fakopp) for 10 destructively sampled *E. cloeziana*.

Table 1.10. Veneer predicted stiffness (using the BING acoustic tool) at three different positions along veneer length; coefficient of determinations between veneer stiffness and standing tree acoustic wave velocity measure using a Fakopp.

Parameter	Veneer positions			
	Inner core	Middle core	Outer core	Average
MoE (MPa)	9655	11217	12934	11324
R^2 with standing tree acoustics	0.48	0.59	0.76	0.73

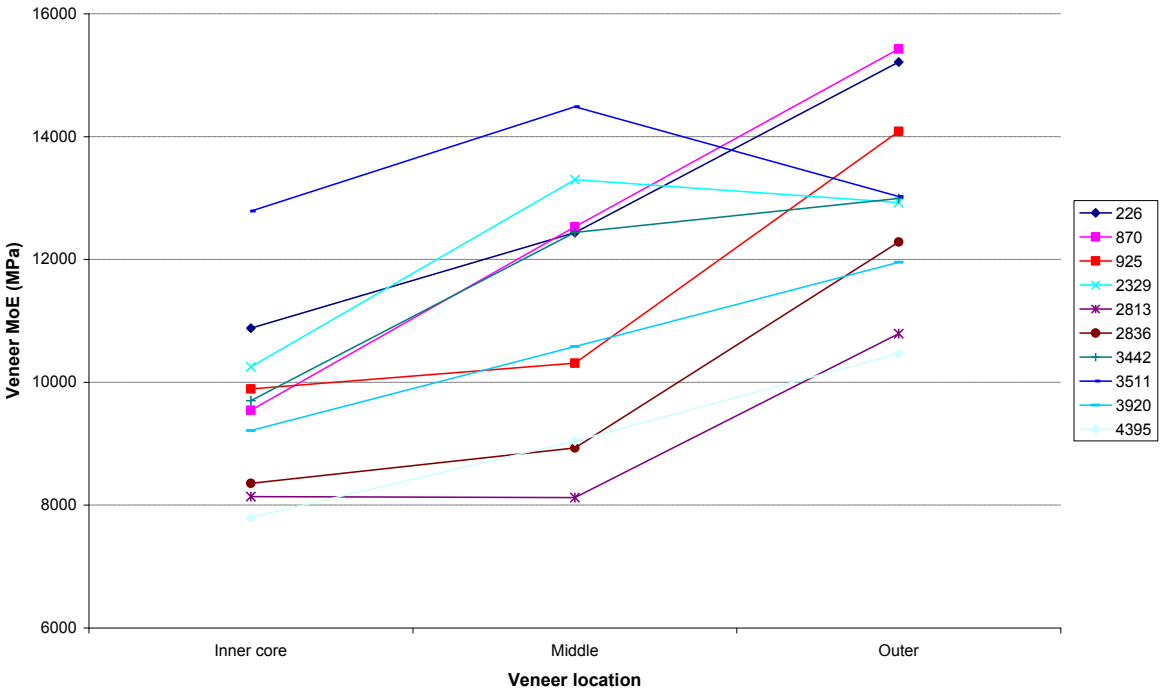

Figure 1.8. Veneer stiffness assessed by the BING device at three positions in 10 families of *E. cloeziana*.

1.4.2.3 Selection of superior E. cloeziana trees for grafting

Based on the results from this study, twenty-four trees were selected for grafting. These trees were selected according to the following selection process. A rank score of 1 to 72 (1 = best, 72 = worst) was assigned to each individual for the results based on diameter (cm), height (m), Fakopp acoustic wave velocity (km/sec), Pilodyn penetration depth (mm), weighted whole core basic density (kg/m^3), sapwood width (mm) and heartwood proportion (%). An index that weighted traits according to 'expert opinion' of the importance of each trait for the future wood processing industry was then used as a basis for selecting trees for grafting. The trees selected in the first pass (based on rank) were then inspected and assessed for straightness, height of limiting defect, sweep, stem taper and branching. An overall rank was then given to each tree and the best trees selected for grafting. The aim was to select trees that had fast growth with more than 50 percent heartwood and acceptable form. It was intended to select one tree from each family but due to poor form of trees in some families, this was not possible. The number of trees from any family was limited to a maximum of two trees from four families to reduce the risk of inbreeding in the clonal seed orchard developed as

a result of this study. Details of plus trees selected in each family/provenance are shown in Table 1.11. Details of each tree are presented in Table 1.12.

Table 1.11. Number of plus trees in each family/provenance.

Family	2nd gen		Provenance									Total
	1ec6-007	1ec6-008	Como	Goomborian	Home	Neerdie	Toolara	Veteran	Wolvi	Woondum	Yurol	
x1390					1							1
x1399								2				2
x1404										1		1
x1416						1						1
x1420			2									2
x1427			1									1
x1430									1			1
x1433									1			1
x1449											1	1
x4086	2											2
x4087		1										1
x4124				1								1
x4184									1			1
x4196							1					1
x4202							1					1
x4204					1							1
x4209					1							1
x4219					1							1
x4221								2				2
x4222								1				1
Total	2	1	3	1	4	1	2	5	3	1	1	24

Table 1.12. Details of plus trees for inclusion in a clonal seed orchard.

Plus tree no.	Straightness*	Overall score†	Heartwood proportion (%)‡	Plus tree no.	Straightness*	Overall score†	Heartwood proportion (%)‡
1ec2-024	5+	4-	51.7	1ec2-036	4+	3	57.7
1ec2-025	5+	4	57.3	1ec2-037	5+	4+	56.0
1ec2-026	5+	4-	68.3	1ec2-038	3+	3-	57.6
1ec2-027	4	2+	59.0	1ec2-039	4+	3+	62.8
1ec2-028	4	3-	62.0	1ec2-040	6	4+	49.8
1ec2-029	5	3-	66.5	1ec2-041	5+	4+	50.4
1ec2-030	5+	3+	54.9	1ec2-042	4	3-	56.0
1ec2-031	3	1+	63.7	1ec2-043	5	2	52.0
1ec2-032	4	2+	54.9	1ec2-044	4-	3	64.6
1ec2-033	3+	3	60.2	2ec2-001	6	4-	48.4
1ec2-034	4-	3-	54.6	2ec2-002	4	3-	51.9
1ec2-035	3+	2+	58.0	2ec2-003	3+	2+	54.9

* – On a scale of 1–6 with 1 the worst and 6 the best.
† – A score of 1– 5 with 1 the worst and 5 the best. Form, taper, branching (angle and size) and defects considered
‡ – Following evaluation of the wood property data heartwood percentage was identified as the core wood property trait that should be considered along with volume and form traits. All other wood property traits measures (e.g. stiffness and density) were above thresholds that made the wood suitable for hardwood solid wood production.

1.4.2.4 Implications for future E. cloeziana plantations

The trees selected in this study are being captured as grafts for a clonal seed orchard which will be established in 2012–2013 financial year on industry partner land. Plantations established using seeds from this new clonal seed orchard (expected to be available by 2015) will have increased product production (in the order of 20%), due to better growth, straightness and form, and increased heartwood percentage over plantations established using wild seed. The information from this study will facilitate development of economic models of the potential growth and value of the timber of this species. This work is on-going.

1.4.3 *Corymbia* hybrids

This study is the first comprehensive overview of the variation in wood properties of the *Corymbia* hybrids.

Two hundred and ten trees across three experiments (Expt 469d HWD near Amamoor – 90 trees, 394a HWD at Devils Mountain, near Sexton – 40 trees and Expt 394b HWD at Mt McEuan, near Hivesville – 80 trees were selected for non-destructive standing tree assessment. There were 30 genetic *Corymbia* hybrid lines and two pure species (Table 1.13) evaluated in the study. Where possible, five trees from each family at each site were sampled to provide a robust evaluation of the taxon. For the pure species comparisons ten *C. citriodora* subsp. *variegata* (CCV) trees were selected in Expt 394b HWD and seven *C. torelliana* (CT) trees were selected in Expt 394a HWD. The selection of *Corymbia* hybrids was based on family volume index growth at each site. In addition deliberate selections were made to ensure that there were linkages between sites and families; four families are common across all three trials, five families common to Amamoor and Devils Mountain, five families common to Devils Mountain and Mt McEuan and four families common to Amamoor and Mt McEuan. The ages at which the measure and sampling was undertaken is presented in Table 1.14.

Table 1.13. Number of families in each taxon sampled in the wood characterisation study.

Taxa*	No. of families
CT×CCV	20
CT×CCC	2
CT×CH	4
CT×CM	4
CCV	1
CT	1
Total	32

* CT = *C. torelliana*, CCV = *C. citriodora* subsp. *variegata*, CCC = *C. citriodora* subsp. *citriodora*, CH = *C. henryi*, CM = *C. maculata*,

Table 1.14. Parameters measured, date and age of measure and age at sampling – *Corymbia* hybrids.

Expt	Measure		Sampling*	
	Date	Age	Date	Age
Expt 469d HWD	April 2010	8.8 years	April 2010	8.8 years
Expt 394a HWD	August 2009	6.6 years	April 2010	7.3 years
Expt 394b HWD	July 2009	6.6 years	April 2010	7.3 years

* Non destructive and destructive sampling was done at the same time

1.4.3.1 Non-destructive evaluation

The heartwood proportion for *Corymbia* hybrids averaged 19% (range 0–40%) at the tree level. The means for the family level are shown in Table 1.15 and Figure 1.9. These values are relatively low when compared to other species; however, the measured trees were about eight years old and it is therefore expected that heartwood proportion will develop further as trees grow older. It is noted that the heartwood percentage of two CT×CCV families and two CT×CH families was equal to or greater than that of the pure CCV. This may be due to the fact that the hybrid families were 1.5 years older than the pure CCV and that the trees were from different sites. Given this the finding that some *Corymbia* hybrid families have larger portions of heartwood at a young age may allow the selection of hybrid families and clones that produce larger volumes of heartwood.

At the tree level the weighted whole core basic density averaged 619 kg/m^3 (range 486–755 kg/m^3). There is a large variation between families in basic density (Figure 1.10). It is interesting to note that for the families common to all three sites, basic density is higher at the Mt McEuan site (the driest site) even though these trees were 1.5 years younger than those at Amamoor. This is consistent with other studies that have found higher wood density is associated with slower growth (Pinkard et al. 2010). It should also be noted that the CCV was from Mt McEuan and therefore the whole core basic density of this may be higher (based on this study) than similar aged CCV at the other two sample sites.

Figure 1.11 shows the radial distribution of basic density. At Amamoor and Devils Mountain the basic density was very similar even though the trees at Devils Mountain are younger (age 7.3 years) than those at Amamoor (8.8 years). In general density of each taxa was higher at Mt McEuan (age 7.3 years) than the other two sites. This is consistent with the findings of Pinkard et al. (2010) that slower growth generally results in higher basic density. Comparing variation of basic density across the taxa, CCV had the highest basic density observed in the study and CT had lowest in basic density.

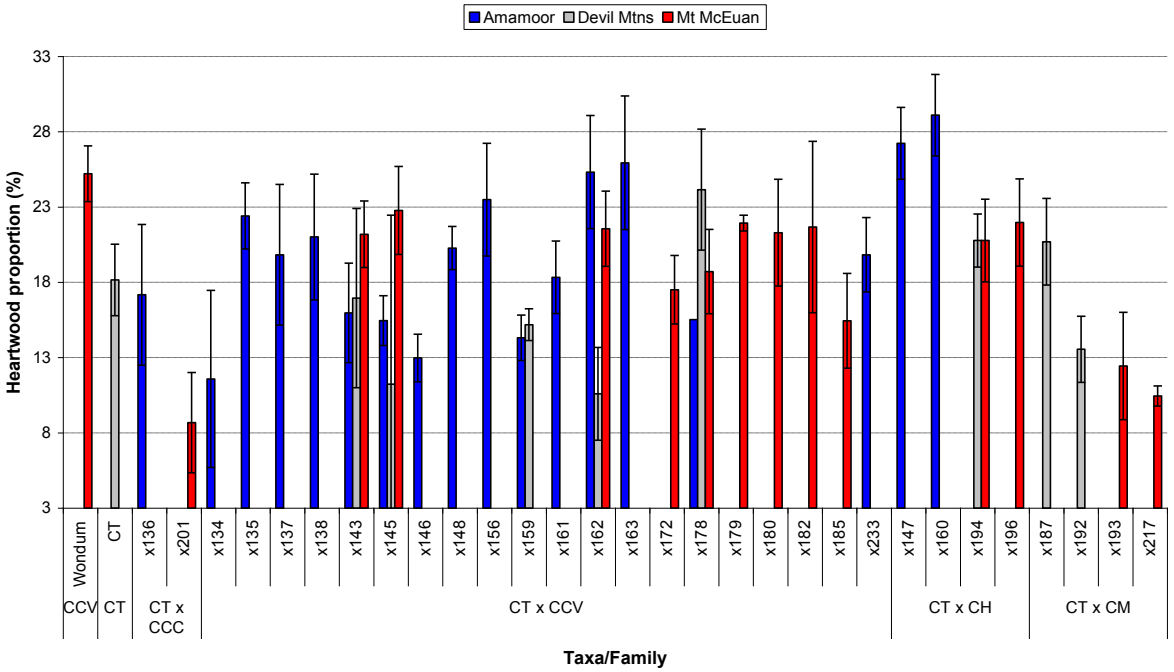

Figure 1.9. Variation in heartwood proportion between families and taxa of *Corymbia* hybrid at three sites. Lines above the bars indicate standard error.

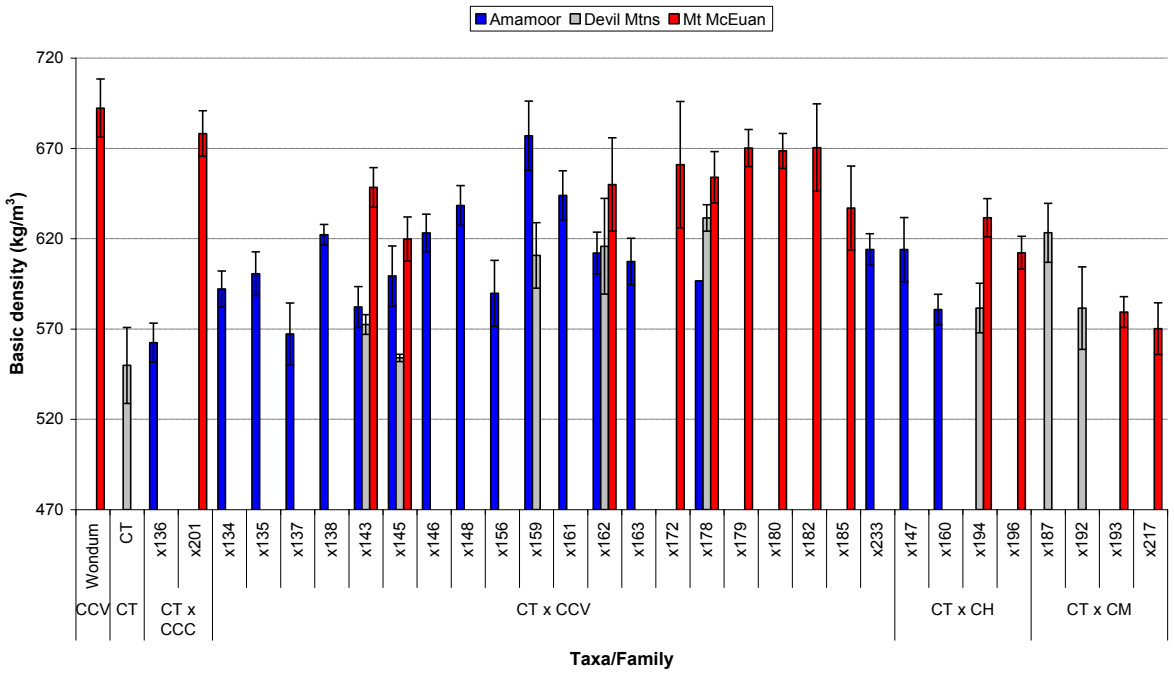

Figure 1.10. Variation in weighted basic density between families and taxa of *Corymbia* hybrid at three sites. Lines above the bars indicate standard error.

Table 1.15. Wood properties of *Corymbia* hybrids.

Expt	Taxon	Family	DBH (cm)	Pilodyn penetration depth (mm)	Acoustic wave velocity (km/sec)	Heartwood ratio (%)	Sapwood width (mm)	Basic density (kg/m^3)		
								Heartwood	Sapwood	Whole core *
469d Amamoor	CT×CCC	x136	16.5	11.6	3.85	17.2	41.7	523	574	562
	CT×CCV	x134	17.4	11.2	3.71	11.6	46.0	529	604	592
		x135	18.5	11.7	4.01	22.4	41.7	560	612	601
		x137	22.9	12.5	3.85	19.8	50.3	494	587	567
		x138	22.7	11.4	4.10	21.0	53.1	542	643	622
		x143	16.0	11.2	3.88	16.0	39.9	511	596	582
		x145	19.0	12.1	4.13	15.5	46.6	545	609	599
		x146	17.7	11.2	4.02	13.0	49.9	569	632	623
		x148	21.5	10.4	3.68	20.3	48.0	561	659	638
		x156	19.6	11.8	3.87	23.5	41.4	542	605	590
		x159	21.0	10.3	3.96	14.3	52.7	596	690	677
		x161	22.3	10.5	3.55	18.3	50.9	576	659	644
		x162	21.6	11.6	3.93	25.3	45.3	560	630	612
		x163	24.0	11.4	3.84	25.9	48.2	556	629	607
		x178	19.9	11.7	3.85	15.5	53.1	542	608	597
		x233	19.0	11.2	3.92	19.8	44.1	541	632	614
		Average	**20.2**	**11.3**	**3.89**	**18.8**	**47.4**	**549**	**626**	**611**
	CT×CH	x147	23.8	10.8	3.86	27.2	44.9	563	633	614
		x160	25.2	11.9	3.89	29.1	44.7	531	603	581
		Average	**24.5**	**11.3**	**3.87**	**28.2**	**44.8**	**547**	**618**	**597**
394a Devils Mountains	CT	**Average**	**17.7**	**13.8**	**3.32**	**18.2**	**43.2**	**538**	**554**	**550**
	CT×CCV	x143	15.0	10.3	3.85	17.0	37.1	514	584	573
		x145	22.1	12.0	3.67	11.2	60.7	551	554	554
		x159	15.9	11.9	3.56	15.2	40.3	546	622	611
		x162	19.3	10.7	3.95	10.6	57.1	569	624	616
		x178	21.6	11.3	3.78	24.2	45.3	579	648	632
		Average	**18.8**	**11.2**	**3.77**	**15.7**	**48.4**	**557**	**616**	**606**
	CT×CH	x194	24.3	11.4	3.85	20.8	54.2	528	596	582
	CT×CM	x187	19.4	11.4	3.75	20.7	44.0	585	633	623
		x192	19.7	10.7	3.74	13.5	52.6	520	591	582
		Average	**19.5**	**11.1**	**3.75**	**17.7**	**47.6**	**558**	**616**	**606**
394b Mt McEuan	CCV	**Average**	**16.7**	**9.3**	**4.06**	**25.2**	**37.2**	**671**	**699**	**692**
	CT×CCV	x201	16.5	9.3	3.75	8.7	51.6	682	678	678
		x143	16.2	10.8	3.84	21.2	39.5	637	652	648
		x145	18.4	11.9	3.85	22.8	42.2	600	627	620
		x162	17.3	10.5	3.90	21.6	40.8	624	657	650
		x172	16.8	9.9	3.81	17.5	42.2	597	676	661
		x178	17.2	10.7	3.93	18.7	44.0	638	658	654
		x179	15.1	11.2	3.81	21.9	35.5	626	682	670
		x180	16.6	10.4	3.98	21.3	40.7	606	685	669
		x182	18.4	10.0	3.78	21.7	43.0	631	687	670
		x185	16.8	10.7	3.80	15.4	44.5	605	643	637
		Average	**17.0**	**10.7**	**3.86**	**20.2**	**41.4**	**618**	**663**	**653**
	CT×CH	x194	17.3	11.1	3.73	20.8	42.6	623	632	632
		x196	17.8	11.2	3.64	22.0	41.7	587	620	612
		Average	**17.6**	**11.1**	**3.68**	**21.4**	**42.1**	**605**	**626**	**622**
	CT×CM	x193	17.2	12.1	3.57	12.4	47.1	550	584	579
		x217	16.8	12.1	3.66	10.4	49.6	567	571	570
		Average	**17.0**	**12.1**	**3.62**	**11.4**	**48.4**	**559**	**578**	**575**

* Note weighted basic density

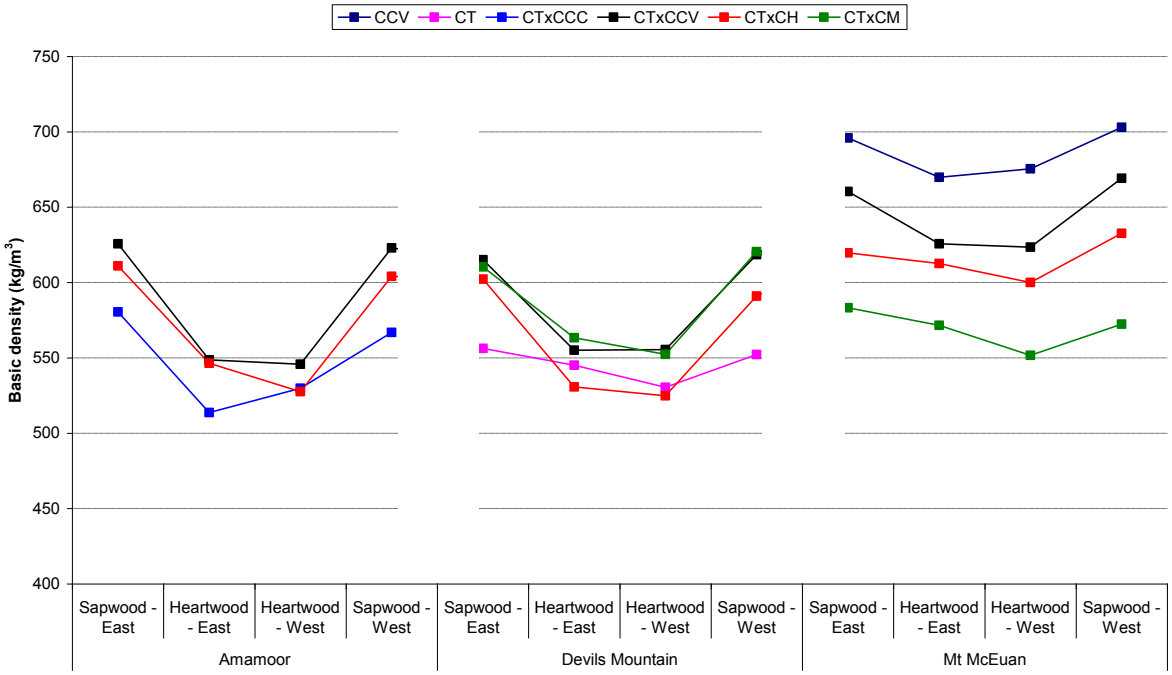

Figure 1.11. Radial distribution of basic density *Corymbia* hybrid taxa samples at three sites.

1.4.3.2 Destructive Evaluation

Plate 1.9. Collecting sample tree measure data – David Lee, Martin Davis and Bruce

Plate 1.10. Barking logs – Tony Burridge and John Huth

A subsample of 20 trees from the Amamoor site was selected for destructive sampling (Plates 1.9 and 1.10). Trees were chosen to represent the best trees based on their tree form and tree size, covering a range of families available. The 20 trees were selected from three hybrid taxa; one family of CT×CCC, 11 families of CT×CCV, and two families of CT×CH. The results of wood properties from those 20 trees are presented in Table 1.16. Figure 1.12 also shows a decreasing trend of variation in heartwood proportion along tree height.

Table 1.16. Wood property results from 20 destructively sampled *Corymbia* hybrid trees.

Parameter	Mean	Standard deviation	Minimum	Maximum
Acoustic sound wave velocity (km/sec)* on the 1.5 m billet	3.4	0.24	2.7	3.8
Static MoE-clear block samples (MPa)	12632	2259	6778	15954
Static MoR-clear block samples (MPa)	105	15	65	130
Basic density – heartwood (kg/m^3)	532	38	464	617
Basic density – sapwood (kg/m^3)	599	47	536	696
Weighted basic density – whole disc (kg/m^3)	584	43	527	680

* Measured by a Director HM200 – an instrument that uses acoustics to measure wood properties of logs

Useful correlations were found between non-destructive standing tree increment core measures and processing values from destructive assessments. Standing tree acoustic measurement correlated strongly (R^2= 0.83) with static bending modulus of elasticity measured on small clear samples (Figure 1.13); there was also a good correlation (R^2= 0.61) between standing tree acoustic measurement stiffness of veneer sheets as measured by BING (Figure 1.14). Pilodyn pin penetration could predict basic density of core samples with accuracy of about 60% (range 45–68% across the three sites; Figure 1.15). This might be useful tool for ranking purposes of selection best trees and families.

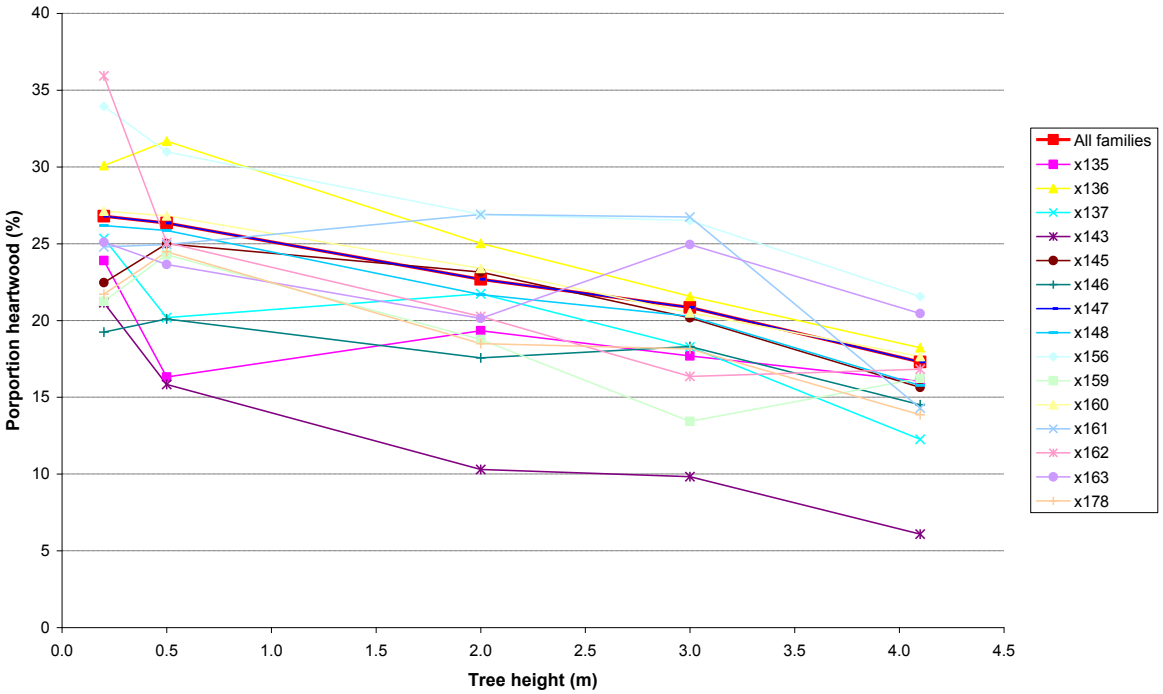

Figure 1.12. Variation in heartwood proportion along tree height for 15 *Corymbia* hybrid families.

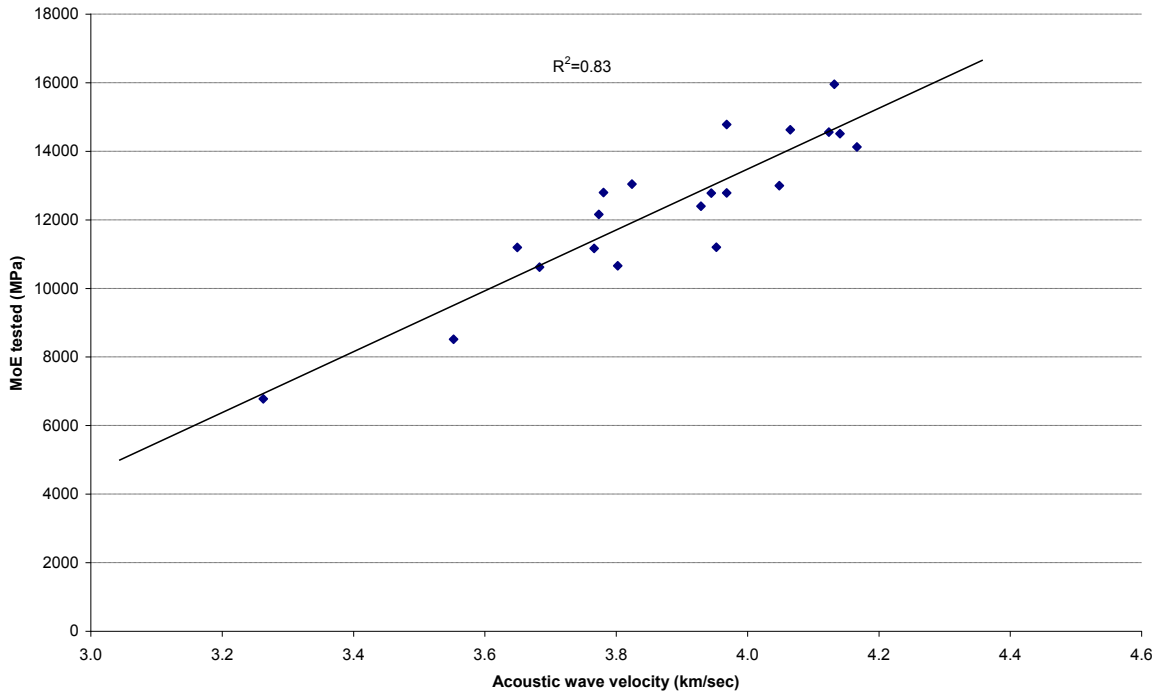

Figure 1.13. Relationship between MOE (from clear sample) and standing tree acoustic wave values for *Corymbia* hybrids.

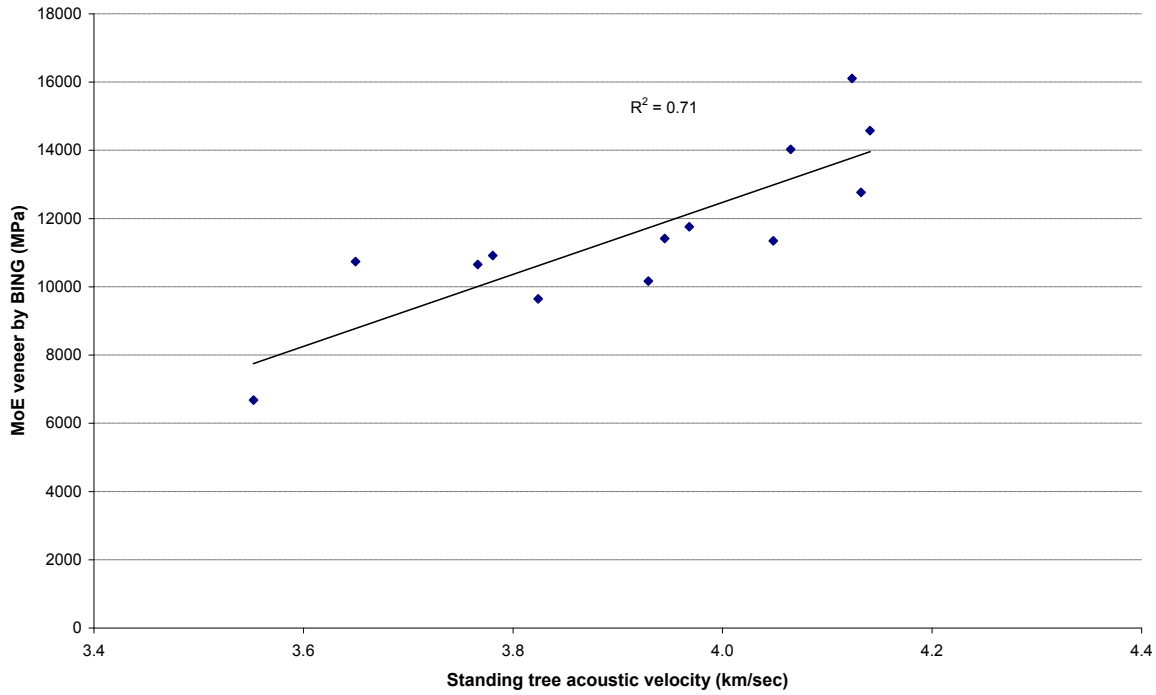

Figure 1.14. Relationship between MOE of veneer samples by BING and standing tree acoustic values for *Corymbia* hybrids.

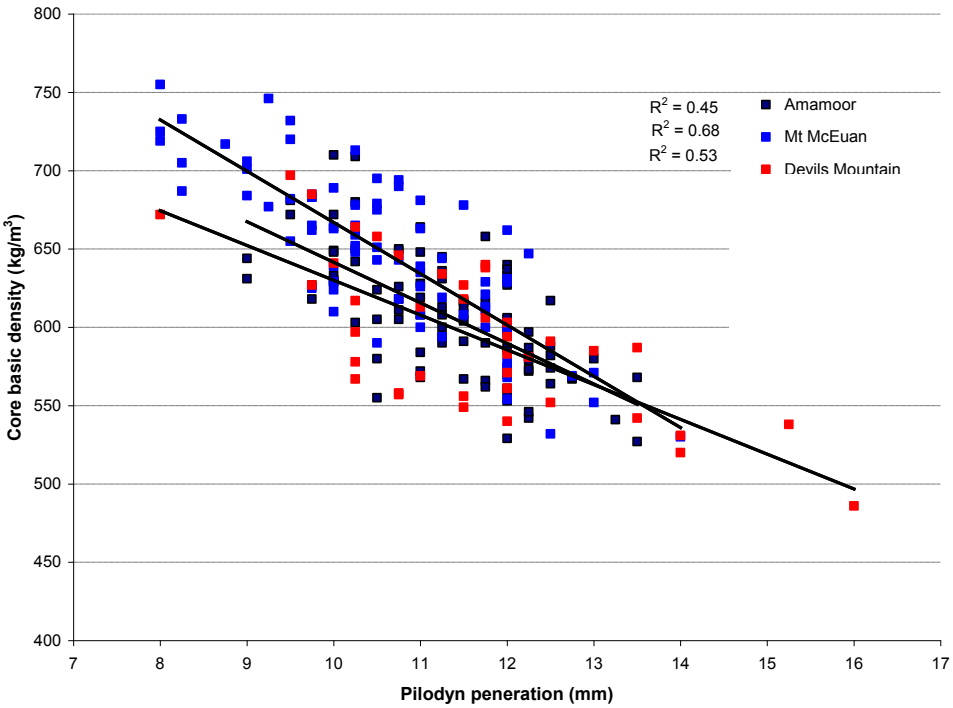

Figure 1.15. Relationship core basic density and Pilodyn penetration depth for *Corymbia* hybrids

1.4.3.3 Selection and commercialisation of elite Corymbia hybrid trees

Based on the growth form and wood characterisation studies undertaken during this project, 60 trees were selected (using a weighted index selection) that combined the attributes of superior volume growth and form and elite heartwood and stiffness characteristics (based on the ranking from the non-destructive and destructive sampling). These 60 elite trees (e.g. clone ctva-124, Plate 1.11) have a broad genetic base with 14 *C. torelliana* and 12 spotted gum (CCC, CCV, CH and CM) parents (Table 1.17). These 60 trees were induced to coppice in August 2011; of these only 15% have coppiced to date due to dry period following coppice induction (Plate 1.12). The recent wet period is expected to result in a large increase in the number of trees that coppice.

A commercialisation agreement has been reached between DEEDI and Clonal Solutions Australia Pty Ltd (CSA) to evaluate these elite growth, form and wood property trees for rooting. CSA will collect the coppice shortly and provide DEEDI with ramets of the better rooting clones for clonal testing on commercial growers land and screening for *Quambalaria* and myrtle rust. Ultimately, a subset of the clones that combine the attributes of having elite growth, disease resistance, form, wood properties and propagation potential will be bulked up under this commercialisation agreement to provide the hardwood plantation sector with a new tranche of elite *Corymbia* hybrid clones that are more stringently tested than the eight commercial clones currently available.

The clones selected from this project should improve plantation productivity (of plantations growing this material) in the order of 30% increased productivity and profitability (conservatively) over that based on unimproved germplasm currently available.

1.4.3.4 Implications for future Corymbia plantations

Plate 1.11. Clone ctva-124

The trees selected as a result of this study are being captured as cuttings for a clonal field and propagation tests in conjunction with Clonal Solutions. These trials will be established in 2012–2014 calendar years as bulked up clones become available. Commercial clones released as part of this study will have significantly increased growth, disease resistance, form, heartwood percentage and straightness of plantations in the order of 30% over that of plantations established with wild pure *Corymbia* species seed. This will result in increased product value and recoveries from plantations established with this germplasm. This study will also facilitate development of economic models of the potential growth and value of the timber of these hybrids. This work is on-going.

Plate 1.12. Coppice shoots – CT x CCV stump

Table 1.17. Wood properties of the 60 *Corymbia* hybrid trees selected for cloning.

Location	Clone name	Family	DBH (cm)	Pilody n penetration	Acoustic wave	Heartwood ratio	Sapwood width	Basic density (kg/m^3)			Stiffness (MPa)
								Heart wood	Sap wood	Whole core	
	ctca-012	x136	19.9	11.5	3.97	27.3	39.2	535	612	591	9307
	ctha-014	x147	25.9	9.0	3.93	30.2	45.0	550	666	631	9742
	ctha-015	x147	22.5	11.0	3.84	26.7	40.2	619	658	648	9549
	ctha-016	x160	24.3	12.0	4.08	20.4	50.7	550	620	605	10079
	ctha-017	x160	31.4	11.8	3.80	30.0	46.2	560	568	566	8183
	ctha-018	x147	21.8	11.5	4.23	30.1	39.8	556	642	616	11013
	ctha-019	x160	21.6	11.0	3.84	37.5	37.0	481	626	572	8429
	ctha-020	x160	26.9	12.3	3.97	28.3	49.6	548	617	597	9401
	ctha-021	x147	25.4	12.3	3.65	31.1	43.7	535	552	546	7273
	ctva-110	x163	25.4	11.5	3.63	26.4	53.5	545	643	617	8129
	ctva-111	x163	23.5	11.3	3.77	29.0	46.7	576	610	600	8512
	ctva-112	x162	20.3	12.0	4.07	24.0	40.3	548	599	587	9700
	ctva-113	x159	22.4	10.3	4.04	14.2	59.9	628	722	709	11574
	ctva-114	x233	23.8	10.0	4.07	25.8	49.1	576	648	630	10410
	ctva-115	x135	16.7	11.3	4.10	25.2	33.4	599	660	645	10834
	ctva-116	x156	18.8	10.0	3.82	29.1	35.4	600	669	649	9491
	ctva-117	x161	25.5	10.0	4.08	22.1	56.6	607	690	672	11195
	ctva-118	x163	26.7	11.0	3.85	32.6	46.5	556	663	628	9290
	ctva-119	x134	26.0	11.3	3.91	31.2	41.5	537	647	613	9390
	ctva-120	x138	23.3	11.5	4.22	29.9	46.3	545	636	609	10842
	ctva-121	x138	21.4	10.8	4.07	21.4	50.7	545	629	611	10097
	ctva-122	x138	21.1	12.0	3.84	30.6	39.7	563	673	640	9431
	ctva-123	x159	22.0	10.0	4.04	14.5	55.5	612	726	710	11591
	ctva-124	x162	18.8	10.3	4.07	19.0	46.2	602	661	650	10741
	ctva-125	x159	26.5	10.3	3.95	19.7	55.6	617	696	680	10624
	ctva-126	x137	24.0	13.3	3.85	35.5	42.6	465	582	541	8003
	ctva-127	x137	23.0	11.8	4.07	21.5	51.1	479	585	562	9325
	ctva-128	x162	27.5	10.8	3.72	24.0	59.7	555	637	618	8572
	ctva-129	x138	23.8	10.5	4.15	11.8	64.3	518	638	624	10744
	ctva-130	x148	22.6	11.3	3.70	23.5	46.1	554	661	636	8724
	ctva-131	x163	22.8	11.3	4.01	32.9	36.2	566	663	631	10137
	ctva-132	x145	22.3	10.5	4.13	17.2	50.1	533	620	605	10331
	ctva-133	x162	23.9	12.5	3.75	39.8	37.6	536	622	588	8279
	ctva-134	x135	23.6	12.5	4.05	25.0	52.1	540	585	574	9408
	ctva-135	x178	24.8	11.0	4.12	13.3	59.5	546	617	608	10339
	ctva-136	x143	21.3	11.5	3.84	27.0	40.0	536	641	613	9033
	ctva-137	x156	23.8	12.3	3.78	32.8	40.5	486	570	542	7747
	ctva-138	x148	23.7	12.0	3.85	20.4	54.6	590	649	637	9423
469d Amamoor	ctva-139	x138	24.1	12.0	4.21	11.4	64.7	542	638	627	11116
	ctha-022	x194	23.2	10.3	3.94	25.2	40.9	533	645	617	9601
	ctha-023	x194	27.0	10.8	3.94	21.2	63.0	497	574	558	8649
	ctma-001	x187	24.6	10.5	3.77	30.0	44.3	621	674	658	9335
	ctma-002	x192	21.6	8.0	3.99	17.4	50.5	622	683	672	10709
	ctma-003	x187	20.0	9.8	3.82	19.5	43.1	644	695	685	9979
	ctva-152	x178	27.1	10.8	3.81	32.0	50.2	586	674	646	9375
	ctva-153	x159	25.3	10.3	4.04	16.0	66.3	565	683	664	10840
394a Devils Mountain	ctva-154	x162	26.1	9.8	4.30	18.4	65.8	553	644	627	11599
	ctca-013	x201	22.5	8.0	3.82	20.3	54.8	733	723	725	10602
	ctva-140	x182	19.6	9.3	3.85	26.7	44.2	706	760	746	11036
	ctva-141	x162	16.1	9.5	4.04	25.3	33.6	713	722	720	11754
	ctva-142	x145	20.2	11.0	4.02	21.1	50.0	617	645	639	10348
	ctva-143	x172	17.5	10.3	4.04	25.5	38.3	559	684	652	10644
	ctva-144	x178	19.5	10.3	4.05	28.1	40.0	650	689	678	11113
	ctva-145	x182	18.7	9.0	3.72	30.0	33.1	622	735	701	9724
	ctva-146	x162	17.3	9.3	4.12	24.6	40.6	608	699	677	11512
	ctva-147	x182	24.2	10.0	3.67	30.8	45.3	585	698	663	8929
	ctva-148	x145	20.2	12.0	3.83	28.9	38.9	589	646	629	9234
	ctva-149	x180	18.5	10.5	4.02	31.8	35.6	582	671	643	10371
	ctva-150	x180	16.8	9.8	3.92	26.6	35.0	649	695	683	10504
394b Mt McEuan	ctva-151	x143	19.8	10.3	3.94	22.9	45.6	649	662	659	10255

* Note weighted basic density

1.4.4 *E. pellita*

Trees for this study were selected from Expt 767a ATH located near Ingham. Although the trees had suffered wind damage due to Tropical Cyclone Charlotte on 12 January 2009 and some trees had up to 5% lean, it was considered that they were still suitable for this study.

From the measurement results, the genetic resource was divided into four major genetic groupings with a smaller number of sub-groupings (Table 1.18). A total of 180 trees[6], comprising 45 individuals from each of the four major genetic groups were then selected on paper for the field sampling component. The selection process aimed at ensuring that the trees covered the range of diameters classes available. All trees had a lean of between 0–5 degrees.

Diameter and height was measured at age 12.8 years in October 2009.

1.4.4.1 Non-destructive evaluation

Non-destructive sampling was done in November 2009 at age 12.9 years.

Table 1.19 shows family average tree growth and standing tree and core samples wood properties of the selected families. On average heartwood ratio was 62.8% (range 56% to 70%) and weighted basic density for the whole core was 567 kg/m^3 (range 553 kg/m^3 to 620 kg/m^3).

In general, at the provenance level (ignoring generation) the heartwood percentage of trees from Queensland was lower than that of Papua New Guinea or West Irian Jayan origin (Figure 1.16). The reverse occurred for whole core basic density with north Queensland provenances generally having higher density (Figure 1.17). These two contrasting wood properties of the different provenance regions of the species may provide the opportunity to hybridise north Queensland material with Papua New Guinea and West Irian Jayan germplasm to increase both density and heartwood percentage of the resulting germplasm. The radial basic density is presented in Figure 1.18.

[6] It was planned to only select 80 trees for the NDE sampling and about 20 trees for the destructive sampling under this project. However, as this species was also included in the PHRF Composite and in the SFAQ project this allowed us to sample 180 trees in total.

Table 1.18. Summary of *E. pellita* trees sampled for NDE.

Genetic type	Genetic group	Origin	Provenance	No. individuals
1st generation seed orchard	North Queensland seed orchard	Papua New Guinea	Ggoe	9
			Keru	5
			Tokwa	9
		West Irian Jayia	Muting	11
			Bubul	11
	Total			**45**
	Melville Is seed orchard	Papua New Guinea	Ggoe	7
			Keru	10
			Tokwa	2
		West Irian Jayia	Bubul	5
			Kumaf	8
			Muting	13
	Total			**45**
Unimproved Natural collections	Papua New Guinea	–	Ggoe	5
			Kiriwo	20
			Serisa	20
	Total			**45**
	North Queensland	Northern provenances	Daintree	11
			Mossman	5
			Julatten	6
		Central provenance	Kuranda	15
		Southern provenances	Abergowie	4
			Cardwell	11
	Total			**45**
Total number of trees sampled				**180**

Table 1.19. Tree growth, standing tree and core wood properties origin/family averages for *E. pellita.*

Origin	Family	DBH (cm)	Pilodyn pin penetration (mm)	Acoustic wave velocity (km/sec)	Heartwood proportion (%)	Basic density (kg/cm^3)		
						Heartwood	Sapwood	Whole core*
West Irian Jayia	Bubul	27.1	13.8	4.0	66.0	520	627	567
	Kumaaf	22.9	15.3	3.9	63.3	526	602	565
	Muting	27.6	14.2	4.0	67.9	516	613	561
	Average	26.6	14.3	4.0	66.5	519	616	564
Papua-New Guinea	Ggoe	25.8	15.2	3.8	67.3	500	597	547
	Keru	25.8	14.8	3.9	61.7	501	600	553
	Kiriwo	25.2	14.8	3.9	65.9	505	602	556
	Serisa	26.2	14.1	3.7	64.6	505	606	558
	Tokwa	23.4	14.7	3.8	65.2	504	598	554
	Average	25.5	14.7	3.8	65.1	503	601	553
Queensland	Abergowie	25.7	12.1	4.0	58.6	587	657	620
	Cardwell	24.3	12.9	4.0	58.9	503	629	567
	Daintree	28.5	12.6	3.7	63.3	505	613	561
	Julatten	28.4	11.3	3.7	56.5	533	663	593
	Kuranda	28.9	12.7	3.8	57.0	525	623	568
	Mossman	28.1	13.2	3.7	61.1	536	634	584
	Average	27.3	12.5	3.8	58.6	525	633	577

* Note weighted basic density

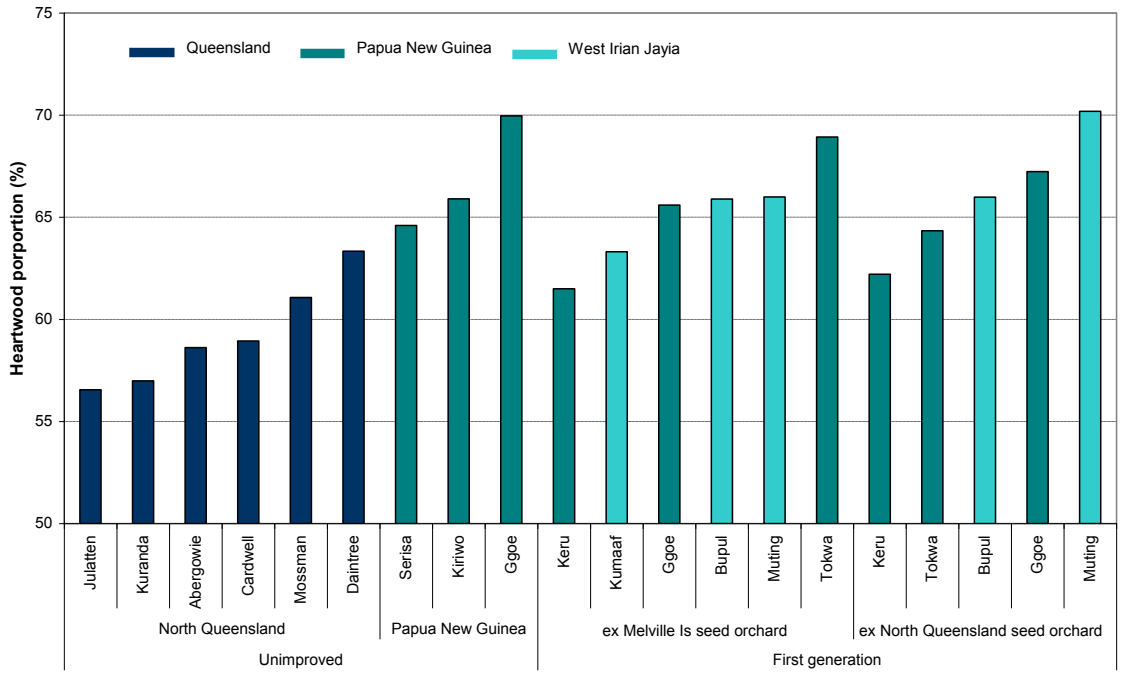

Figure 1.16. Heartwood proportion (%) in *E. pellita* from a number of genetic sources.

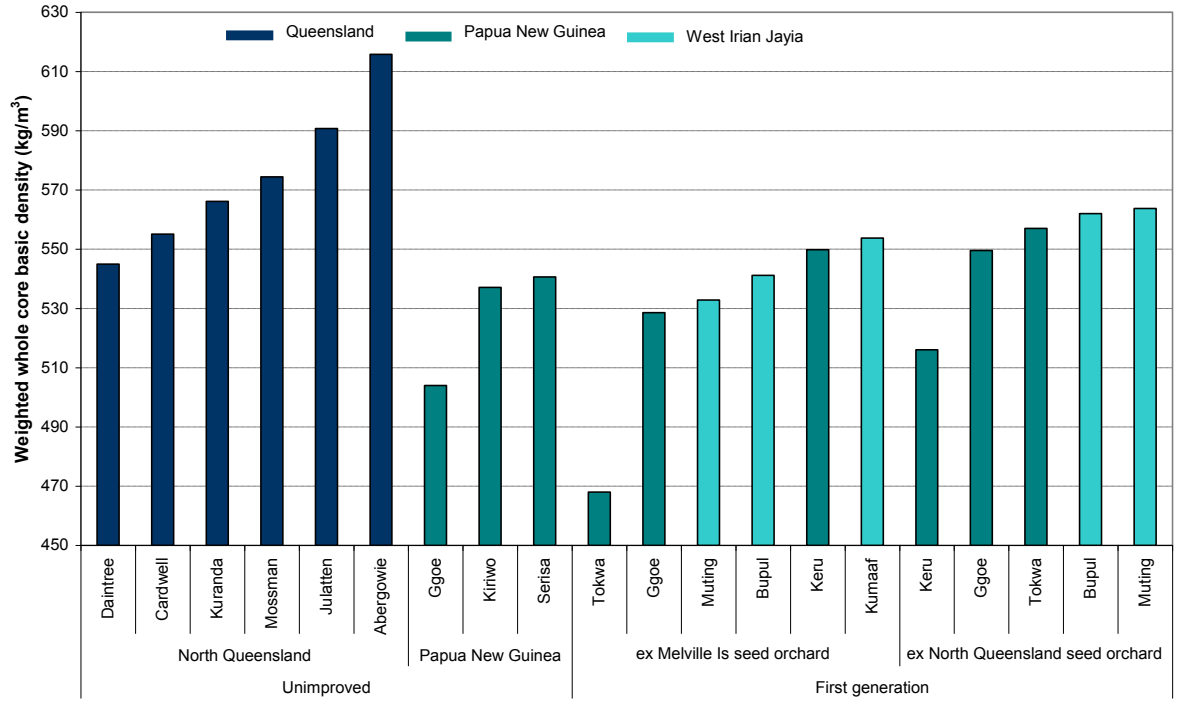

Figure 1.17. Weighted whole core basic density of *E. pellita* from various sources.

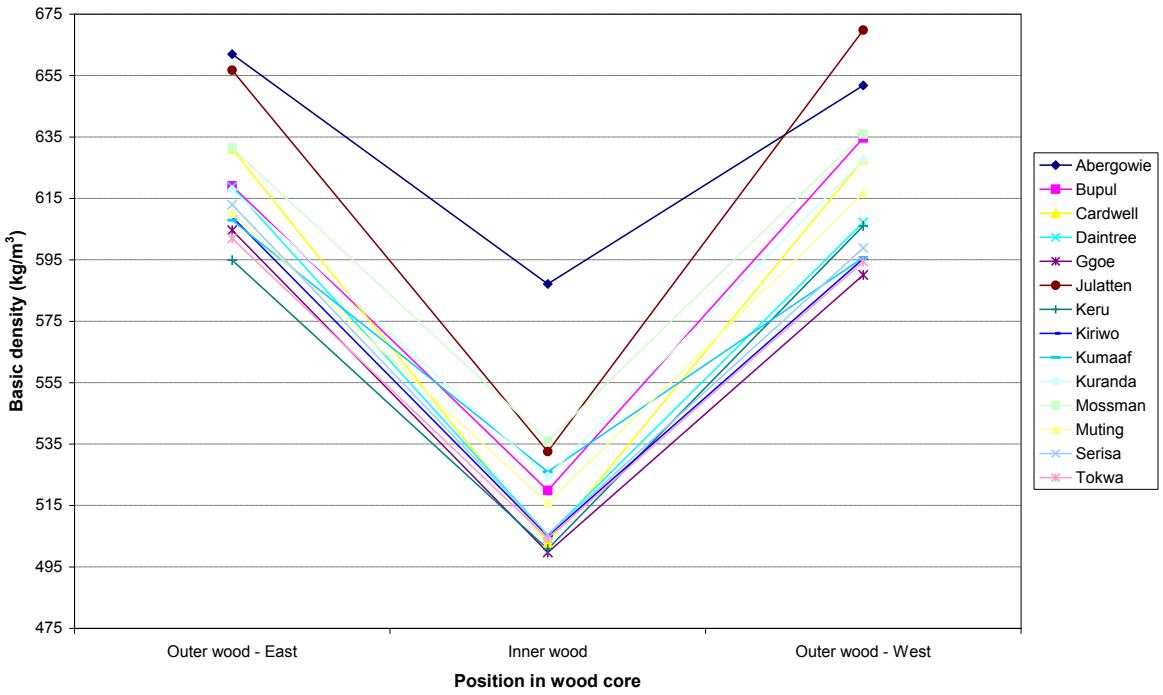

Figure 1.18. Radial basic density (kg/m^3) in the wood core sample of *E. pellita* from 14 different provenances.

The average basic density values for the unimproved material and the improved material from the two Australian improved genetic sources is shown in Table 1.20. For all provenances the density of the inner wood was higher than that for the outer wood (Figure 1.18).

Table 1.20. Weighted whole core basic density (kg/m^3) of unimproved and improved sources of *E. pellita* from Papua New Guinea and West Irian Jayia.

Provenance	Weighted whole core basic density (kg/m^3)		
	Unimproved	First generation	
	Papua New Guinea	Ex Melville Is	Ex Queensland
Ggoe	504	529	550
Serisa	541	–	–
Kiriwo	537	–	–
Keru	–	550	516
Bupul	–	541	562
Kumaaf	–	554	–
Muting	–	533	564
Tokwa	–	468	557

1.4.4.2 Wood colour

Plate 1.13. Core samples of *E. pellita* after seven days exposure to ultraviolet light

A sample of *E. pellita* cores exposed to seven days ultra violet light is shown Plate 1.13. There was a general trend for the heartwood in the Queensland provenances to be higher in a* value, which represents richness in red colour, than that in the unimproved Papua New Guinea and West Irian Jayia provenances (Figure 1.19).

Heartwood of Queensland provenances had also lower L* value, which represents lightness, suggesting that Queensland wood is darker than Papua New Guinea and West Irian Jayia provenances (Figure 1.20).

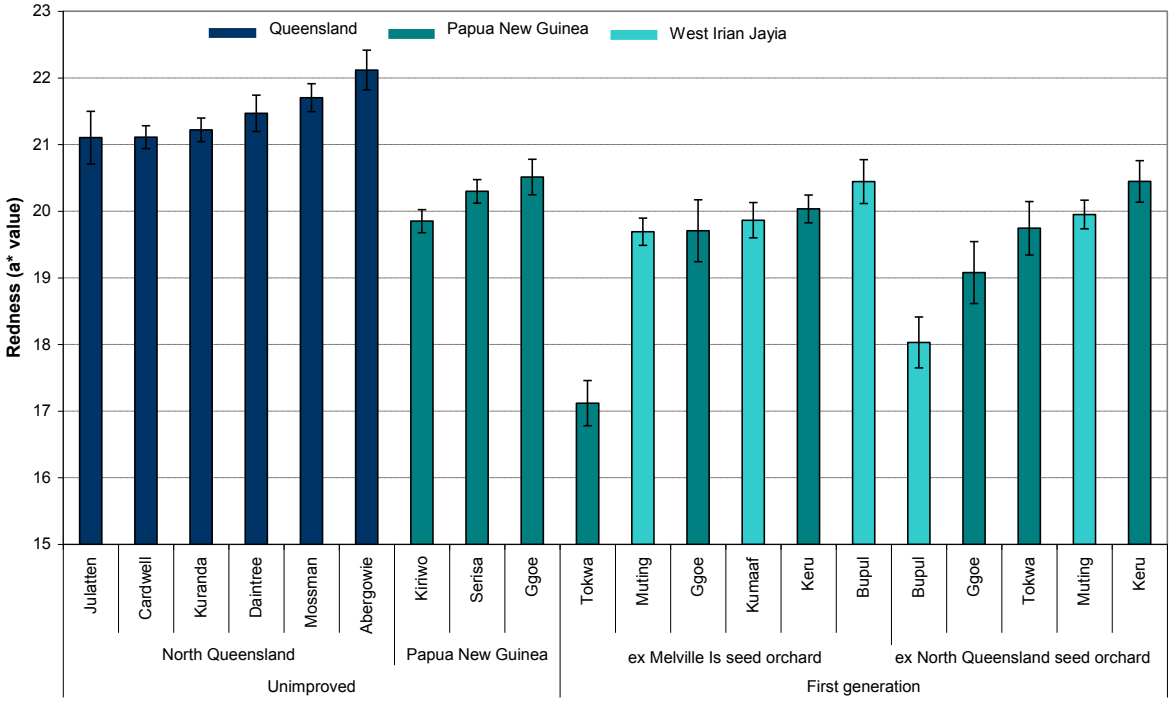

Figure 1.19. Redness a* value of *E. pellita* (higher value = redder).

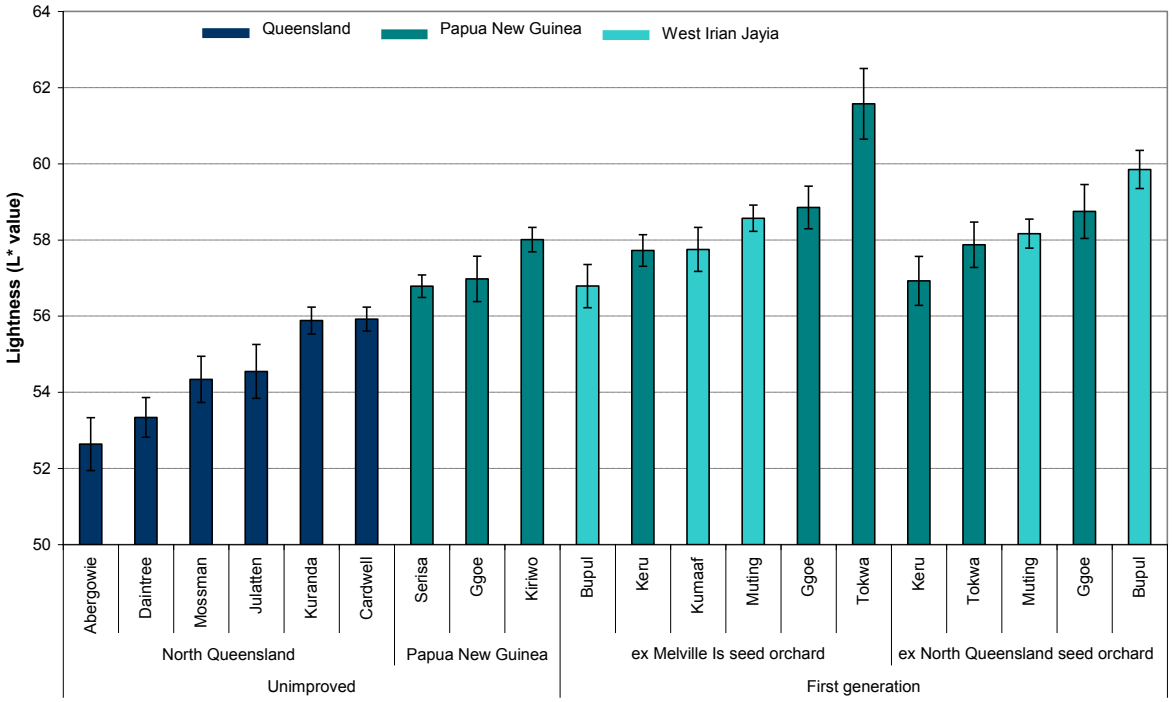

Figure 1.20. Lightness L* value of *E. pellita* (higher value = lighter colour).

1.4.4.3 Destructive evaluation

Thirty-eight trees were destructively sampled at age 13.5 years in May 2010 and peeled using a spindleless lathe. The selection was based on tree form and wood properties variation, while aiming to include the widest structure in families and provenances possible. Table 1.21 provides results for wood properties assessment on the disc samples taken above the peeling billet.

Table 1.21. Wood property results from 38 *E. pellita* trees destructively sampled.

Parameter	Mean	Standard deviation	Minimum	Maximum
Sapwood width (mm)	24.3	6.0	14.5	44.2
Heartwood proportion (%)	57.8	7.4	36.7	76.9
Basic density – outer heartwood (kg/m^3)	637	56	518	737
Basic density – sapwood (kg/m^3)	623	52	502	727
Weighted basic density – whole disc (kg/m^3)	615	48	503	701
Radial unit shrinkage (12% to 5% moisture content)	0.24	0.04	0.11	0.34
Tangential unit shrinkage (12% to 5% moisture content)	0.34	0.04	0.21	0.42

The predicted MoE (acoustic velocity^2 × basic density) is shown in Figure 1.21.

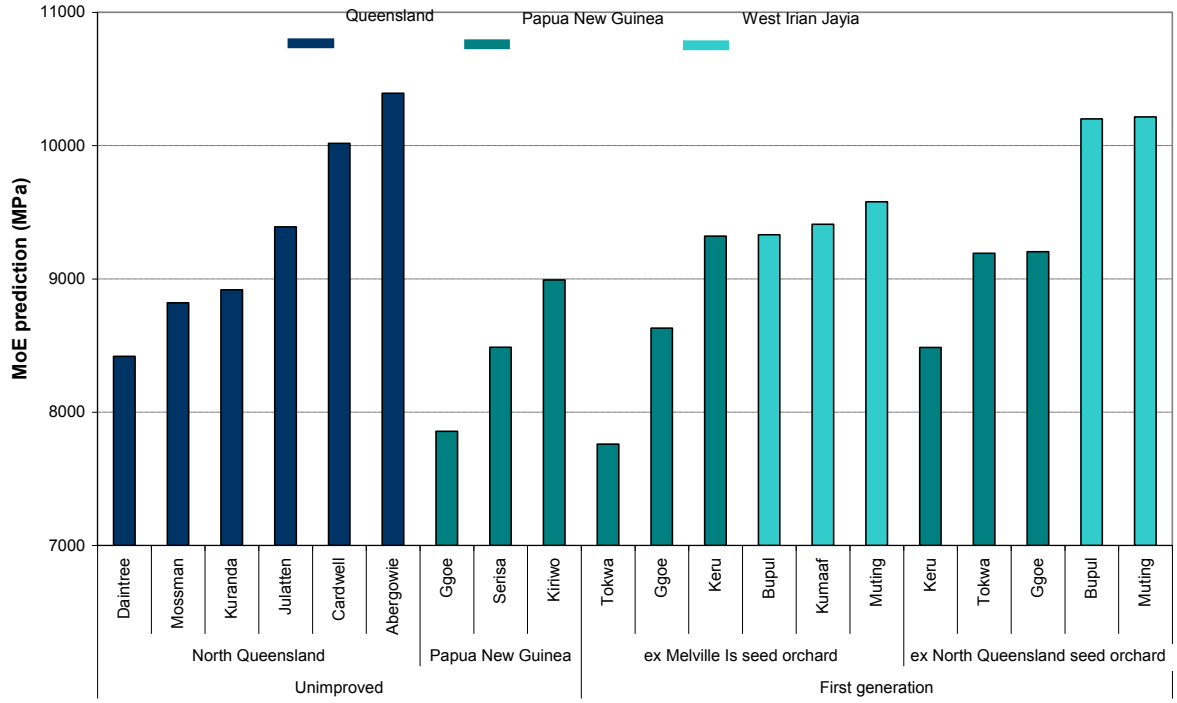

Figure 1.21. Predicted MoE (acoustic velocity^2 × basic density) of *E. pellita* provenances.

1.4.4.4 Variation in heartwood proportion with tree height position

As the *E. pellita* study benefited from three projects contributing to the study, we were able to evaluate the heartwood proportion of *E. pellita* trees up to approximately 7 m. There was a gradual decrease of heartwood percentage up the tree. In most provenances, heartwood proportion at 2.6 m ranged between 55 and 70%. The Jullaten (north Queensland) provenance had the lowest heartwood at 2.6 m (40.6%) and the Bupul (West Irian) provenance at the highest heartwood proportion (85%) (Figure 1.22).

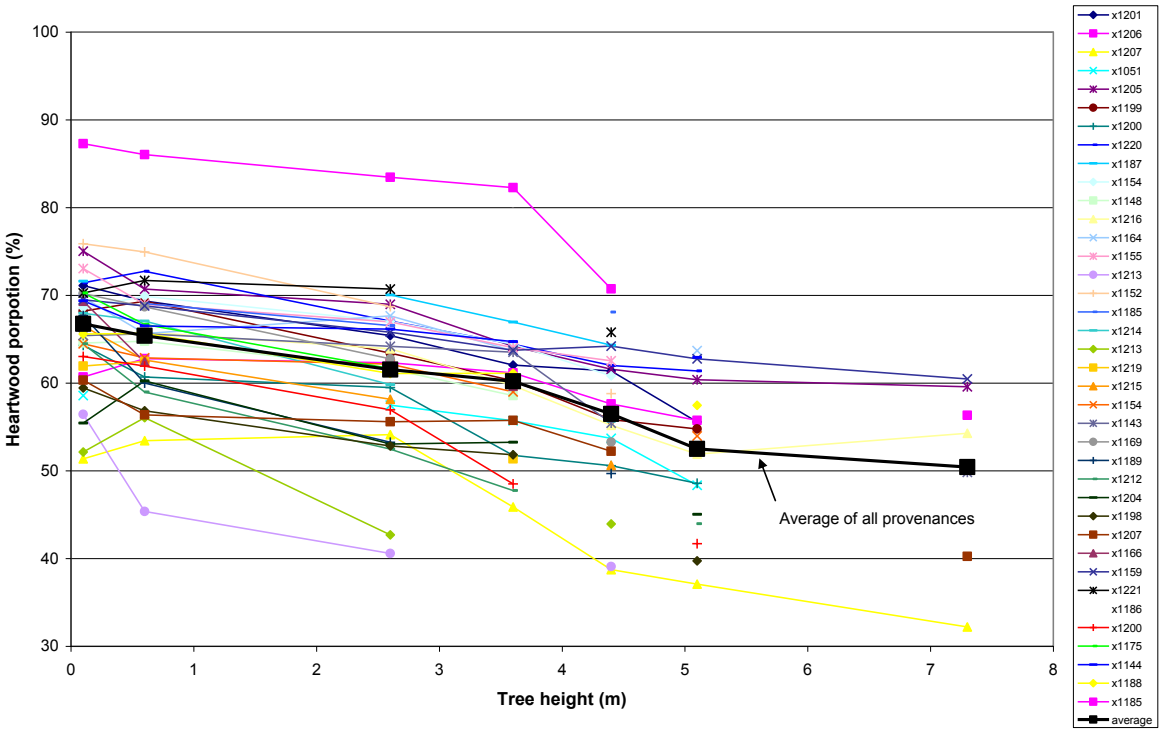

Figure 1.22. Heartwood proportion (%) to 7.3 m in *E. pellita* from 14 different provenances.

1.4.4.5 Implications for future E. pellita plantations

Unfortunately, the trial Expt 767a ATH, the source of the trees evaluated in this study, was severely damaged by Cyclone Yasi in January 2011 and the industry partner who was planting *E. pellita* in north Queensland (Elders Pty Ltd) lost their entire *E. pellita* plantation (approximately 3,000 ha) to the same cyclone. Based on this, it was not possible to capture any elite trees based on growth, form and wood property. If a new plantation grower emerges that wishes to plant *E. pellita* in Queensland's wet tropics, then new trials can be established using seed in stock. It will still be possible to benefit from the information captured in this study to develop *E. pellita* trees with large proportions of heartwood, desirable colour and wood density. Currently further work on this species has been suspended until / if a new plantation grower emerges who wants to grow *E. pellita*.

1.5 Identification of elite trees with superior wood characteristics, growth and form

This project over-achieved on its target to select 60 elite trees. Instead, 108 trees were identified with superior growth, form and wood characteristics (*Corymbia* and *Eucalyptus*; Table 1.22). Of these, commercialisation agreements are in place for 60 superior *Corymbia* hybrid trees, and clonal seed orchards (CSOs) are being developed for *E. argophloia* and *E. cloeziana*. Clonal seed orchards of the latter two taxa will be established on project-partner land in the next 18 months when the grafted trees reach sufficient size to survive as planted trees. This component is on-going.

All the elite germplasm identified in this project will be screened either directly (*Corymbia* hybrid clones) or indirectly (progeny trials from seed of the elite *E. argophloia* and *E. cloeziana* trees selected in this project) for resistance to myrtle rust. The *Corymbia* hybrid clones will also be screened for *Quambalaria* resistance. This will be on-going work over the next few years as material becomes available.

Table 1.22. Number of elite trees selected based on superior wood characteristics, growth and form of each project taxon.

Taxon	No. trees evaluated for wood analysis	No. of elite trees selected
E. argophloia	81	24†
E. cloeziana	72	24†
Corymbia hybrids	210	60
E. pellita	80	Trial damaged by cyclone Yasi
All taxon	**443**	**108**

* in partnership with Clonal Solutions Australia

† grafting for a clonal seed orchard is on-going

1.6 Summary and conclusions

The four taxa evaluated for wood properties in this project all showed great promise for veneer production with sufficient variation found to allow the selection of outstanding trees with superior growth form and wood properties. The Corymbia hybrids had a mean basic density of 614 kg/m^3 compared to approximately 800 kg/m^3 for plantation / native forest derived trees. This is equivalent to 77% of the native forest derived trees at approximately eight years old (Table 1.23). Similar aged *E. cloeziana* had 73% of its native forest basic density. The other two taxa (*E. argophloia* and *E. pellita*) were evaluated at age 13 years. These two species had 85% and 72% of their respective mature native forest basic densities. The available published data indicates that further increases in

basic density required to reach mature values is important, and it is reasonable to suggest that this may be reached around the age of 25–30 years, which is close to the final harvest age for a sawlog management regime.

Comparisons of other traits with mature plantation grown trees / native forest trees are not possible as this is the first study of many of these wood properties in Australia.

The most common species for the manufacture of structural plywood in Australia is radiata pine. On average it has a veneer MoE of about 10,400 MPa (combining inner and outer veneer samples). The average results of MoE on all species from this report were superior to that of radiata pine. This is an important and valuable attribute for the Queensland plantation hardwood resource, as the resulting ply or timber of all four taxon will be stronger for the same piece size, than ply or timber derived solely from *Pinus* species.

Table 1.23. Comparative performance of each taxon and basic density that of mature grown trees.

Species	**Age**	**Basic density (kg/m^3)**		
		Mean whole disc young plantation	**Mature plantation (age, years)**	**Native forest**
Corymbia hybrids*	7.5–8.5	614	802[†] (40)	800[‡]
E. argophloia	13.0	725	838[§] (32)	855[#]
E. cloeziana	7.5	594	769** (46)	810[††]
E. pellita	13.0	567	Not available	790[††]

* Compared to CCV here, as no wood property data on mature Corymbia hybrids is available.

[†] Leggate W., Palmer G., McGavin R. and Muneri A. (2000).

[‡] Queensland Forest Service (1991).

[§] Armstrong M. (2003).

[#] DPI (undated)

**Muneri A., Leggate W., Palmer G. and P. Ryan (1998).

[††] Bootle, K. R. (2005).

Chapter 2. Optimal propagation systems for elite *Corymbia* and *Eucalyptus* germplasm

2.1 Systems for capturing elite *Eucalyptus* germplasm

Research efforts for capture of elite varieties were directed towards the two project species considered difficult-to-capture as rooted cuttings from coppice, *E. cloeziana* and *E. argophloia*. Efforts were directed at developing grafting systems for capture of selected varieties of these species into the nursery. The two other project species, *C. citriodora* (and hybrids) and *E. pellita*, were considered relatively easy to capture. Successful grafting systems had been developed by DEEDI researchers in recent years for capture of *Corymbia*, while *E. pellita* is inherently easy to capture as rooted cuttings from basal coppice.

Fundamental to tree genetic improvement is the ability to identify elite germplasm that will underpin breeding programs, providing genetic gain in subsequent generations. Seed that is made available through such programs is expected to improve the value of plantations by increasing biomass production and/or superior wood properties. Improved seed can be produced from selectively thinned un-pedigreed stands or progeny trials to retain the best individual trees – thereby creating seed production areas and seedling seed orchards respectively. However, greater gains can theoretically be made in clonal seed orchards by incorporating only the very best parent trees (potentially from multiple sites), thereby increasing selection intensity for a given trait.

Other components of this project added value to conventional breeding efforts by characterising the timber resource of subtropical and tropical *Corymbia* and *Eucalyptus* species in seed orchards and other plantings, allowing selection of trees with desirable wood properties as well as good growth and form. Clonal seed orchards based on this material will deliver superior seed to commercial growers; however, success depends not only on being able to select the parent trees but also being able to capture and multiply this material by methods of vegetative propagation.

This study investigated several specific issues in vegetative propagation of two key plantation species – *E. cloeziana* and *E. argophloia,* in order to optimise germplasm capture protocols. The aims were:

1) to investigate the effects of IBA concentrations on cuttings survival and root formation in *E. cloeziana*;
2) to evaluate the importance of genetic compatibility between rootstock and scion of *E. cloeziana* in the nursery success rates and graft union integrity of older clones;
3) to investigate the storage potential of scion material kept under cool moist condition; and
4) to evaluate a new method of managing *E. argophloia* grafts in the glasshouse to increase capture rates of this difficult to propagate species.

2.1.1 Source materials

Material was sourced from trials in southern Queensland (Table 2.1). In the case of *E. cloeziana*, trees with the best growth and form were selected initially, as wood property studies had yet to be concluded. However, individuals with desirable wood properties were subsequently included in the program as results became available. Material of *E. argophloia* was collected from the best individuals growing in a nearby progeny trial.

Table 2.1. Location of trials from which material was sourced for this project.

Taxa	Experiment No.	Location
E. argophloia	460c HWD	Kilkivan
E. cloeziana	481d HWD	Cpt 56 St Mary's LA SF 57 St Mary
E. cloeziana	537 HWD	Yurol State Forest, Pomona

2.1.2 Experiment 1: Producing rooted cuttings of E. cloeziana from stump coppice.

2.1.2.1 Methodology

Plate 2.1: Stump coppice of *E. cloeziana* approximately 3.5 months after felling trees

Eucalyptus cloeziana trees from a 12 year old fertiliser trial (537HWD) were felled in August 2010 for destructive sampling as part of a wood properties study. A large percentage of felled trees produced stump coppice (Plate 2.1). On 8 November 2009 seven coppicing stumps were given 40 litres of water with 5 grams of Thrive fertiliser to improve the health and vigour of material for this study. Weed growth around the stumps was controlled with glyphosate herbicide (360 grams a.i.).

Whole coppice shoots were collected from the selected stumps on the morning of 11 December 2010, bundled in wet newspaper and transported to the Gympie glasshouse facility in an insulated container where the coppice was soaked in 1% sodium hypochlorite solution for 60 seconds and segmented into double-node cuttings. The leaf area of each cutting was reduced by approximately 50% and the cuttings then immersed in Benlate™ fungicide.

The cuttings were dipped in Rootex™ rooting powder at a concentration of either 1, 3 or 8g per kg indole-3-butyric acid (IBA) powder or pure talc (control treatment) before being set into Hyko trays containing a propagation mix of 70% pine bark and 30% perlite with slow release fertiliser, hydrated lime, Micromax™, gypsum, and wetting agent added. The cuttings were set in single cell plots as a replicated randomised incomplete block design and misted for 10 seconds every 10 minutes for nine weeks, and 15 seconds every 30 minutes for an additional three weeks before assessment. Cuttings were also treated fortnightly with a foliar application of Fongarid™ fungicide.

2.1.2.2 Results

IBA had a modest effect in inducing root formation in *E. cloeziana*. Whilst the highest IBA concentration (8 g / kg) had the greatest effect in inducing root formation, the mean rooting potential was very low at ~10% (Figure 2.1). Individual clones had maximum rooting potential of between 5% and 15% (data not shown). Intermediate levels of IBA did not appear to differ significantly from the control treatment in their potential to improve rooting.

The number of cuttings still alive after 12 weeks was relatively high and did not differ significantly between treatments. Of this material, nearly half had produced callus but not roots (Figure 2.2). Whilst potentially indicative of latent rooting potential, the actual likelihood of this material producing roots is quite low without additional treatments.

The mean number of primary roots produced on rooted cuttings did not differ significantly between concentrations of rooting hormone. Between one and two roots per cutting were produced, compared with around four primary roots per cutting observed on seedling-derived rooted cuttings (Trueman, this report).

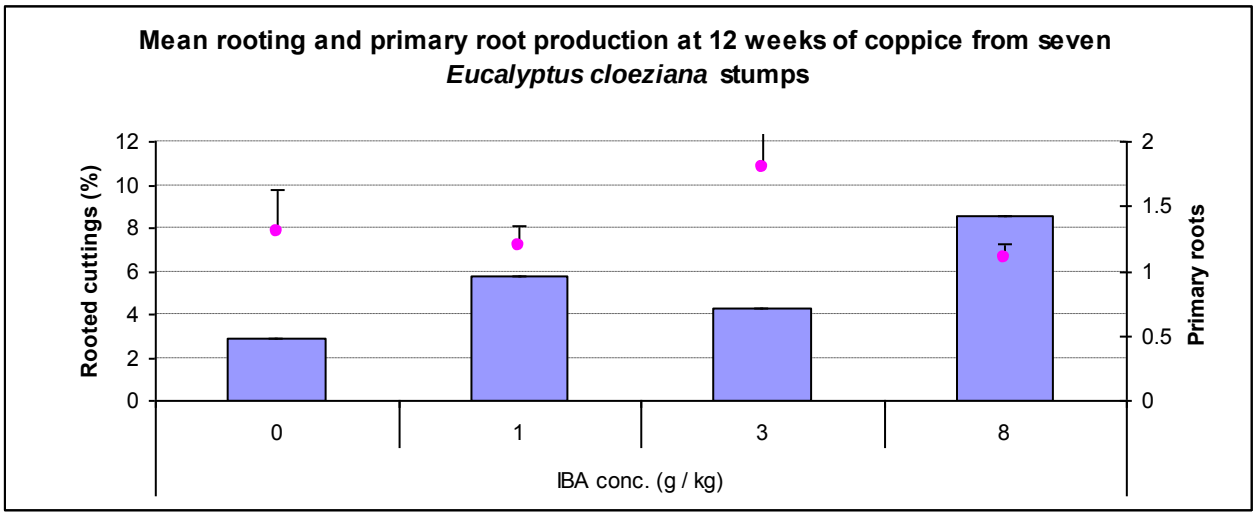

Figure 2.1. Mean rooting (columns) and primary root production (scatter plots) of *E. cloeziana* cuttings taken from cut stumps.

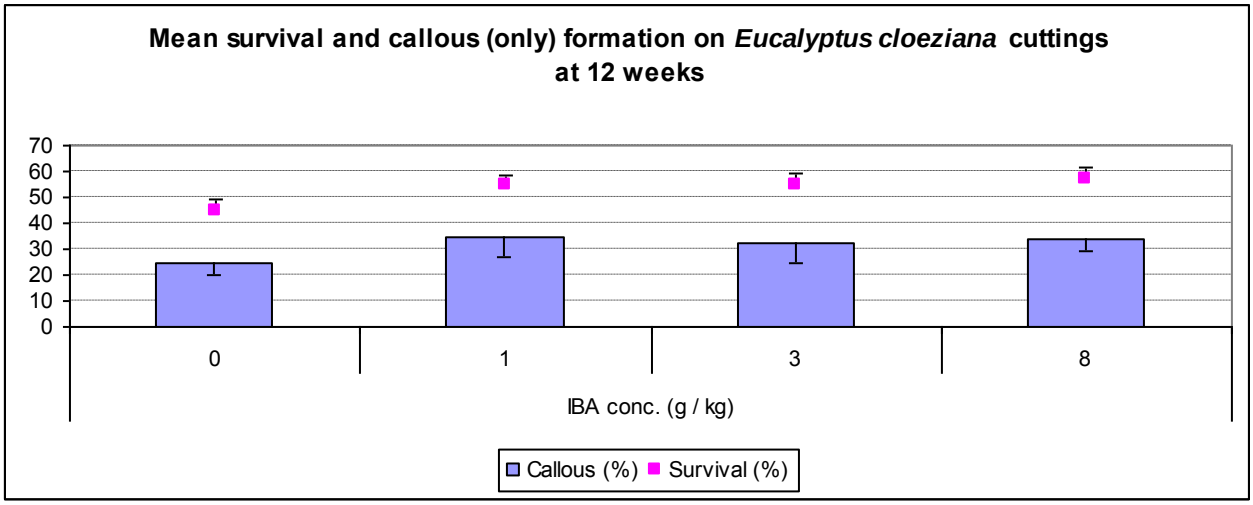

Figure 2.2. Mean survival and percentage of *E. cloeziana* cuttings with callus formation without roots.

2.1.3 Experiment 2a: Genetic compatibility of rootstock and scion in E. cloeziana

2.1.3.1 Methodology

Three rootstock treatments were used in this experiment to investigate the importance of the degree of genetic association between rootstock and scion to successful graft union and to graft vigour. The treatments were as follows: own seed source; half sib seedling; and unrelated provenance (Goomboorian). Rootstock from Ravenshoe provenance (North Queensland) was originally included in this study but was excluded when the seedlot failed to germinate. Seven unrelated trees were selected for grafting from the St Mary's seedling seed orchard (Expt. 481.D HWD) near Tiaro, 60 km north of Gympie. Selections were initially made of the highest ranking individuals, based on phenotypic assessment only (growth and form) and then cross referenced with seed availability for rootstock treatments to identify candidates for this grafting study (Table 2.2).

Table 2.2. Original *E. cloeziana* candidate 'plus' trees for grafting, based on phenotypic selection.

Plot	Row	Tree	Entry	Provenance	Seedlot (mother)	DBH (cm)	Height (m)
107	3	5	215	Veteran	k5675-1	26	21.5
70	2	3	192	Toolara	k5652-1	24.7	21.3
41	6	3	141	Mungy	10823-186	23.2	23.1
28	2	3	9	Veteran	4355-9	26.2	18.1
44	4	3	216	Veteran	k5676-1	24	20.9
51	1	5	183	Wolvi	k5643-1	24.1	19.9
36	6	5	133	Cannidah	10822-173	22.6	21.8

Seed for rootstock was sown into Hyko trays in September 2009. A standard potting media was used consisting of composted pine bark, pine bark fines, slow release fertiliser, hydrated lime, micro elements, gypsum, and wetting agent. The seedlings were raised at the Gympie glasshouse facility until they reached an appropriate size for grafting (approx. 5 months). Most seedlots had poor germination and so a second sowing of seed was required at the end of September to ensure adequate rootstock was available.

Six trees were included in the final selection based on seedling rootstock availability and vigour and grafting commenced on the 11th February 2010 (Plate 2.2a). Scion from a single selected tree was collected in the late afternoon each day, moistened in a plastic bag and transported to Gympie in a cooled insulated container where it was stored overnight in a cold room for grafting the following day. Twenty top cleft grafts were done per treatment per tree using slightly lignified scion material approximately 4 nodes in length (Plate 2.2b), with three replications. The graft union was placed above two leaf pairs of a healthy root stock plant where possible.

a) b)

Plate 2.2. a) Grafting of *E. cloeziana* grafts at Gympie research facility carried out by Paul Warburton (CSIRO) and John Oostenbrink (DEEDI); b) Scion is approximately 2mm diameter and 4 nodes in length.

The grafts were placed under misters (Plate 2.3) in a completely randomised block design and misted for 10 seconds every 10 minutes. As with the cuttings, grafts were treated with an occasional foliar application of fungicide. Graft mortality was initially assessed at 5 weeks, as was the presence/absence of humidity-induced stem and leaf galling (Plate 2.4). Grafts were then moved to a hardening off facility where misting was reduced to 10 seconds every 15 minutes for 12 days after which they were relocated to a shaded bench and irrigated three times per day. A second assessment was done nine weeks post-grafting, after which time the grafts were moved into full sun to harden before being potted into 2.8 l black plastic pots. These grafts were maintained for 12months before undertaking a final assessment of survival and scion rejection (Plate 2.5).

Plate 2.3. New *E. cloeziana* grafts under overhead misting

Plate 2.4. Humidity-induced gall formation on the union and scion stem of an *E. cloeziana* graft

Plate 2.5. Swelling at the union of a 12 month old graft is a recognised sign of genetic incompatibility.

2.1.3.2 Results

Both the 'own' and 'half-sib' seedling rootstock of Family 141 had very poor survival in the glasshouse (21% and 5%, respectively) (Plate 2.6). As this was unrelated to grafting success per-se and had the potential to confound results, these data were omitted as outliers.

Plate 2.6. High mortality of seedling rootstock of Family 141 from Mungy (left) compared with Wolvi provenance (right)

Humidity gall was prevalent within a few weeks of placing the grafts under misting. There was a strong correlation between the occurrence of leaf galling and early mortality (Figure 2.3) although each family was grafted on different days and this may have had a confounding effect. Bearing this in mind, and the fact that graft mortality cannot be solely attributed to galling, the extent of humidity galling on the grafts dictates that this requires strict attention in an *E. cloeziana* grafting program.

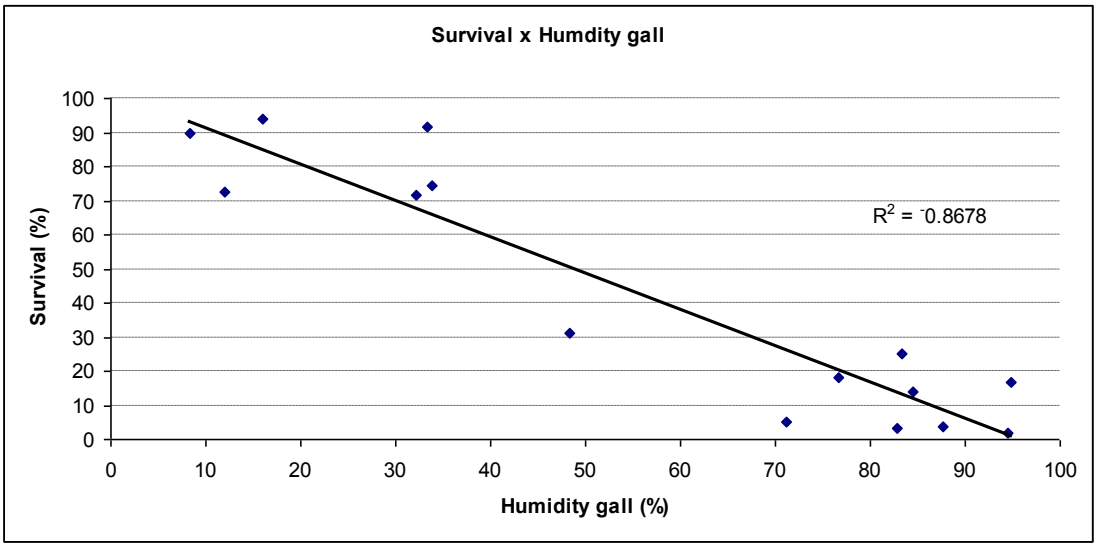

Figure 2.3. Early survival and occurrence of humidity gall on 5 week old *E. cloeziana* grafts.

Table 2.3 shows graft survival and incompatibility. Initial grafting success ranged between 72.2% and 80.9% and, after 9 weeks, this had fallen to between 57.1% and 59.4%. After 12 months there remained little difference in survival between rootstock treatments although it had continued to decline. However, strong differences in expression of graft incompatibility were evident as early as 9 weeks after grafting. Grafts on closely related rootstocks showed less signs of incompatibility than those grafted onto unrelated material. This trend continued throughout the study.

Table 2.3. Mean survival (%) of *Eucalyptus cloeziana* grafts using rootstock of different relatedness (expression of incompatibility (%) are in parentheses).

	5 weeks	9 weeks	52 weeks
Own seedlot	72.2	57.1 (18.3)	36.9 (10.5)
Mother seedlot	80.9	59.4 (22.3)	35.5 (13.2)
Unrelated seedlot	74.7	57.8 (56.2)	33.8 (35.2)

2.1.4 Experiment 2b: Genetic compatibility of rootstock and scion in E. cloeziana: evaluating Ravenshoe provenance.

2.1.4.1 Methodology

A second experiment was undertaken in October 2010 to include the Ravenshoe provenance that failed to germinate in the previous experiment. Due to the proximity of the origin of the candidate trees and the Goomboorian material used as an 'unrelated' provenance, this seedlot was included in the study as a genetically distinct provenance. Four of the previously used candidate trees were included in this study to provide genetic linkage to the first experiment. Seedling rootstock was raised as described in the previous experiment and the same experimental design was used. Survival was assessed at 3, 5, 27 and 60 weeks and incompatibility assessed at 27 and 60 weeks only.

2.1.4.2 Results

Overall, grafting success was lower than that observed in the first experiment – possibly due to seasonal influence. A similar trend of protracted mortality is evident with survival appearing to stabilise around 6 months after grafting (Figure 2.4). There was a small difference in survival between the rootstock treatments but no significant difference in incompatibility between treatments on either assessment date (Figure 2.5). There was,

however, a marked increase in the proportion of grafts showing signs of graft failure as the grafts aged.

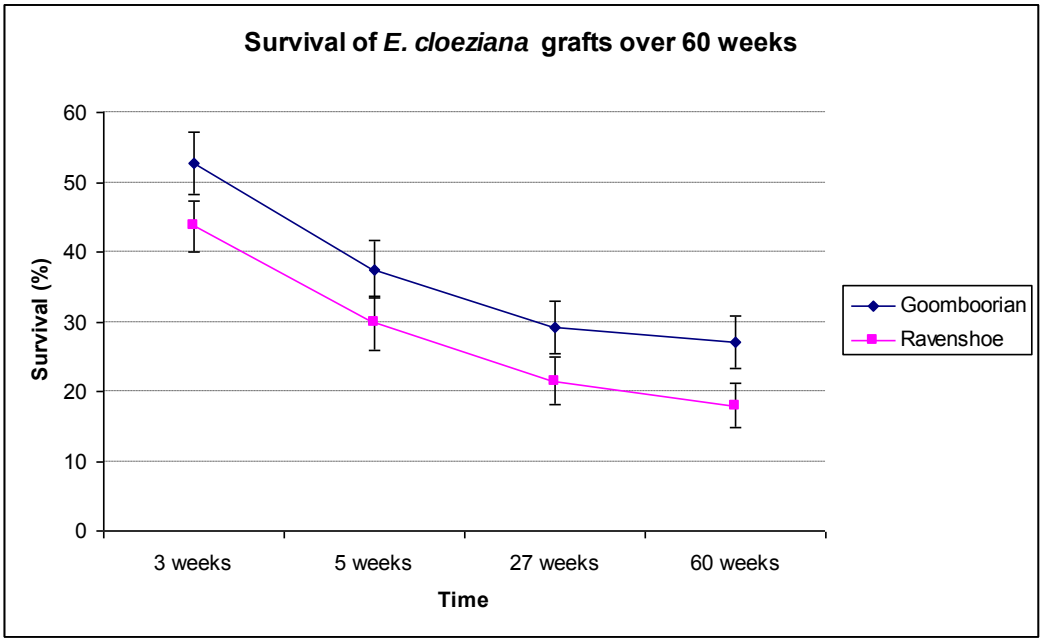

Figure 2.4. Mean survival of *E. cloeziana* clones grafted onto two geographically distinct rootstocks

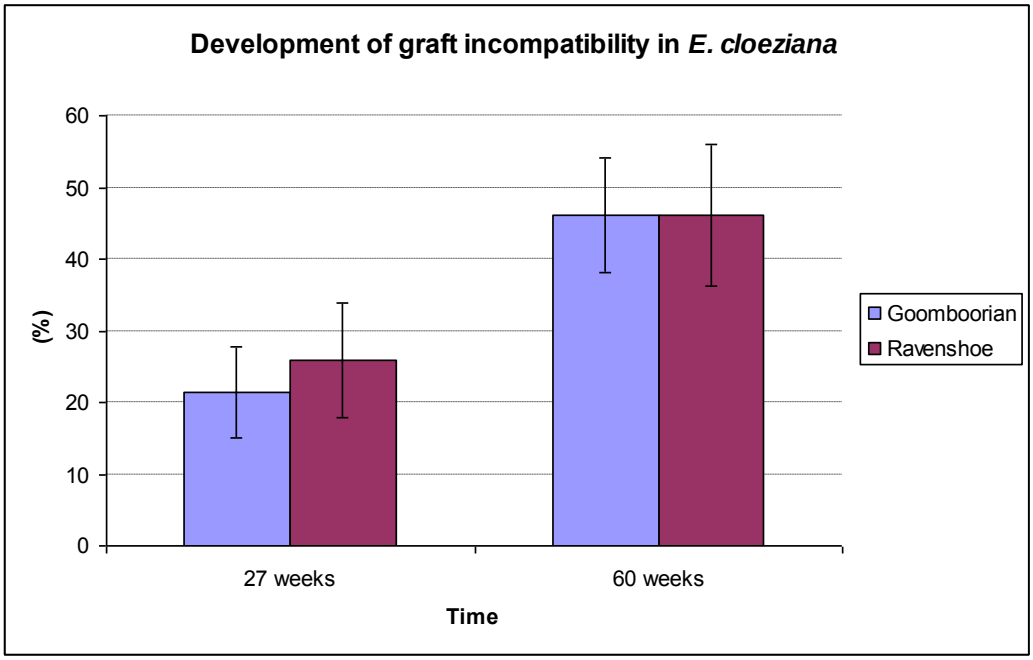

Figure 2.5. Mean incidence of graft incompatibility of *E. cloeziana* clones grafted onto two geographically distinct rootstocks

2.1.5 Experiment 3a: Cold moist storage potential of E. argophloia scion material.

2.1.5.1 Methodology

The effect of cold moist storage of scion material on grafting success of *E. argophloia* was investigated. Seed for rootstock was sown in November 2009 using the standard potting media (described above). The need to re-sow seed, slow germination and poor viability of the second round of seedlings delayed the start of this study until May 2010.

Ten selections were made from a previously un-sourced progeny trial near Kilkivan, Queensland (460cHWD) based on growth and form data from the most recent assessment, done in 2009 (Table 2.4).

Table 2.4 Candidate *E. argophloia* trees, sourced from Kilkivan progeny trial (460cHWD).

Exp ID	Row	Tree	Family	Provenance	% > grand mean (height)	% > grand mean (DBH)
1	2	12	79	Burncluth	227	207
2	3	14	34	Burncluth	174	167
3	3	34	31	Fairyland	170	149
4	4	63	54	Burncluth	227	192
5	4	44	56	Burncluth	184	168
6	2	38	62	Burncluth	160	160
7	6	6	77	Burra Burri	169	174
8	7	18	53	Burncluth	175	179
9	7	22	37	Burncluth	179	164
10	6	22	76	Burra Burri	154	154

Scion material was collected from each individual and divided into subsamples that were either stored in a moistened plastic bags or in a vase containing water, and kept refrigerated at four degrees Celsius for 1, 2, 4 and 8 days. Three grafts were done of each treatment for each candidate tree per storage treatment and placed on heat mats in single plot design within a misted glasshouse. Grafts were misted for 10 seconds every 10 minutes and treated with an occasional foliar application of fungicide.

2.1.5.2 Results

An assessment of surviving plants, undertaken 5 weeks after grafting, revealed 100% mortality.

2.1.6 Experiment 3b: Cold moist storage potential of E. cloeziana scion material.

2.1.6.1 Methodology

The effect of cold moist storage of scion material on grafting success of *E. cloeziana* was investigated in October 2010. Scion material was collected from six individual trees for grafting onto genetically matched rootstock that was raised in the same manner as previously described.

Fresh scion from each selection was divided into three subsamples and stored in moistened plastic bags at 4°C for 1, 2 and 4 days respectively. Fifteen top cleft grafts were done of each candidate tree per storage treatment, with three replications. The grafts were placed under misters in a completely randomised block design and misted for 10 seconds every 10 minutes. Grafts were treated with an occasional foliar application of fungicide and an assessment of survival carried out after 13 weeks.

2.1.6.2 Results

There was a significant interaction between families and days in survival of grafts after 3 months with only two families showing a marked decline in survival of grafts undertaken 4 days after harvesting scion. The remaining families demonstrated no significant decline with duration of storage. The results across families for each day are illustrated in Figure 2.6

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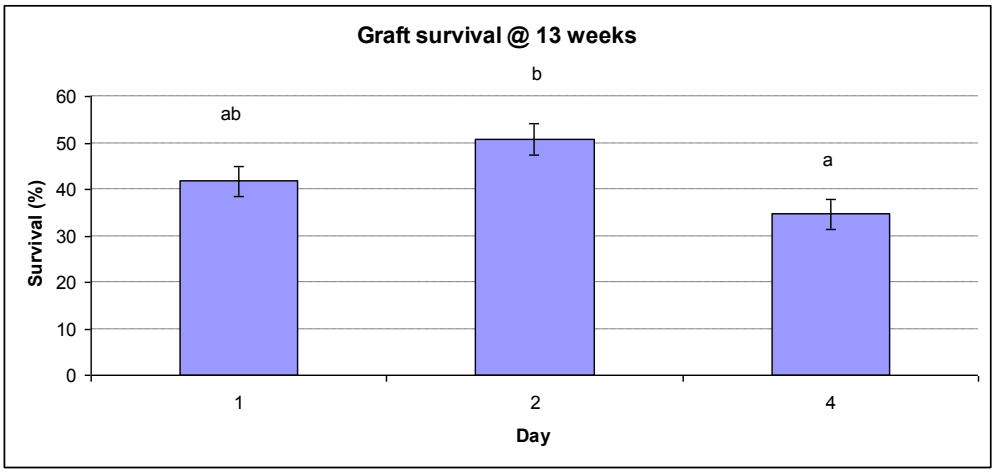

Figure 2.6. Mean survival (%) of grafts of unrelated *Eucalyptus cloeziana* using scion stored in moist cold conditions for 1, 2 and 4 days prior to grafting.

2.1.7 Experiment 4: Evaluating a novel method to increase survival of E. argophloia grafts

2.1.7.1 Methodology

Only six of the selected trees from Kilkivan were included in this study due to the limited availably of suitable grafting material. Rootstock for this experiment was sown in April to ensure that the seedlings were available for grafting in summer. Fifteen grafts per family were done with three replications across two treatments in November 2010. Grafts were placed in a glasshouse in a completely randomised block design, either under a misting regime of 10 seconds every 10 minutes or placed in a bath containing enough water to ensure that only part of the plants' root plug were immersed. Plastic sheeting and shadecloth was erected over the grafts to aid in increasing ambient humidity and reduce photo-oxidative damage to the grafts. Survival was assessed after 5 weeks and the grafts then removed from the glasshouse and placed on a shaded bench where they were irrigated three times per day. A second assessment was carried out after 12 weeks.

2.1.7.2 Results

The difference in survival between the two treatments is presented in Figure 2.7. Despite earlier anecdotal evidence to the contrary, graft survival using the wicking treatment in this experiment was significantly lower than overhead misting. In both cases the survival of grafts at 3 months of age was low relative to when they were initially removed from the glasshouse (at 5 weeks).

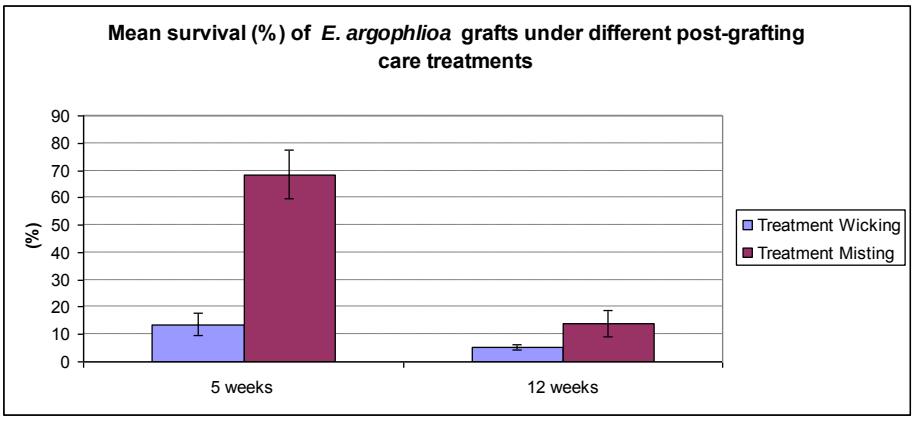

Figure 2.7. Mean survival (%) of *Eucalyptus argophloia* grafts under different glasshouse treatments.

Figure 2.8 shows the family differences in survival at 5 and 12 weeks of grafts maintained under the misting treatment only. There was a significant difference between most families with only two exhibiting survival greater than 25% after 3 months. A number of ramets of each clone have survived beyond 12 months and, typically, there is little evidence of graft incompatibility (Plate 2.7).

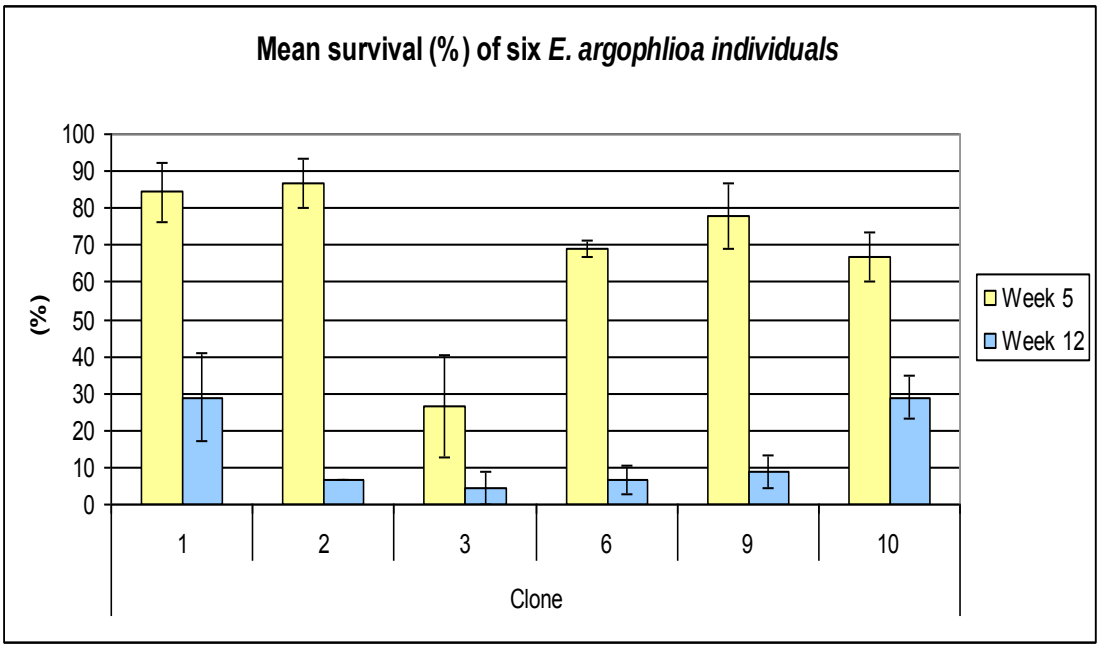

Figure 2.8. Mean survival (%) of grafts of six selected *Eucalyptus argophloia* under overhead misting treatment.

Plate 2.7. Elite *E. argophloia* grafted clone.

2.1.8 Discussion and conclusions: varietal capture methods for Eucalyptus

Previous studies (Baker and Walker 2006; Rodger Peters, Forestry Plantations Queensland Pty Ltd, pers. comm.) have reported low rooting success of *E. cloeziana* cuttings from both coppice and seedling hedges. This study has demonstrated similar findings, confirming that this propagation method is unsuitable for field capture and multiplication of this species. Results show that standard grafting techniques are adequate for capturing elite *E. cloeziana* material although there are several important factors that govern long-term success.

Four-node scion should be grafted onto closely related, vigorous rootstock using a top-cleft located at approximately 2-3 mm stem diameter. Newly grafted plants can be maintained under a misting regime of 10 seconds every 10 minutes although inland provenances (e.g. Mungy) may be sensitive to over-wetting in glasshouse conditions resulting in rapid mortality of the rootstock. Modification to the misting regime by way of slightly shorter misting duration coupled with slightly longer intervals may improve seedling survival. However, inland provenances rarely demonstrate superior performance in pedigreed trials and it is therefore unlikely that large numbers of future selections will be made of trees from these environs.

Humidity-induced galls were present on 37% of all *E. cloeziana* grafts at the time they were removed from the glasshouse. Visual observation of the grafts suggested a possible relationship between the incidence of galls and early graft failure and while mortality cannot be attributed to galling alone, a strong negative correlation exists between galling and mortality. Grafts should be monitored carefully and removed from the glasshouse immediately (if possible) should signs of humidity gall appear. If this is not possible, misting should be altered to a slightly drier regime (e.g. 5 seconds every 15 minutes) and the grafts removed as soon as possible. Plants should ideally remain in the glasshouse facility until bud break but no longer than 5 weeks. Successful grafts should be moved to full sun using the following protocol:

- remove plants from glasshouse and transfer to a shaded facility with good air flow for 12 days where misting is reduced to 10 seconds every 15 minutes,
- move plants to a shaded bench and irrigate three times per day,
- move plants to full sun 9 weeks after grafting.

It is possible that there is a seasonal effect on grafting success although this was not tested with replicated experiments. Best results were obtained in February and it is recommended that grafting be undertaken between late Spring and late Summer when shoot growth is vigorous and ambient temperatures are conducive to rapid plant growth. This also appears

to be of critical importance for *E. argophloia* as grafts undertaken in winter failed despite the use of heat mats under the grafts to improve growing conditions for seedling rootstock.

This study demonstrates the importance not only of pairing scion with genetically matched rootstock to minimise issues of incompatibility, but of retaining grafted ramets for a period of six to twelve months before deployment in the field to allow for delays in mortality and for expression of graft incompatibility to fully develop. The health and vigour of a graft is of critical importance to the integrity of a clonal seed orchard as failed grafts rarely die outright and the (unselected) rootstock is then able to contaminate pollen flow.

E. argophloia has proven difficult to propagate vegetatively. Attempts to induce roots from seedling cuttings and from stump coppice have failed to produce commercially viable rates of success (Ian Last, Forestry Plantations Queensland Pty Ltd, pers com., Baker and Walker 2006). Grafting therefore, remains the best option to capture elite germplasm from the field. Reports of 'wicking' rootstock, rather than overhead irrigation, improving grafting success were not evident in this study. While it is reasonable to expect that grafts may benefit from a slightly drier misting regime given the environment of the species' natural distribution, the need for consistent high humidity remains and this is currently best provided under misting.

While this study has demonstrated family/clonal differences in grafting potential, survival of even the most successfully grafted family still remained low. The survival of grafts at 3 months was low relative to when they were removed from the glasshouse at 5 weeks, indicating potential to refine the process by which grafts are removed from the glasshouse. A staged process similar to that suggested for *E. cloeziana* is recommended.

The process of capturing selected germplasm can necessitate the storing of field material for a short period, particularly when distances between sites and the nursery are great or if a large amount of material is to be grafted. It is helpful therefore to understand the storage potential of scion to avoid wasted effort in grafting non-viable material. Results from this study indicate that, when using appropriate handling storage protocols, material will remain viable for at least 4 days in storage under cold moist conditions.

It has been possible to refine selection-age clonal capture and propagation protocols of *E. cloeziana* and, to a lesser extent, *E. argophloia*. Progress has also been made in capturing elite material of both species for future establishment of clonal seed orchards (Appendix 3). These ramets are maintained at the DEEDI research facility in Gympie where additional ramets can be produced. Future grafting will capture additional elite trees, identified from pedigreed trials, to be incorporated into the breeding program of these species.

2.2 Optimised protocols for producing rooted cuttings of Corymbia and Eucalyptus

Hardwood plantations in southern Queensland are concentrated in regions with relatively inexpensive land and mean annual rainfall less than 1000 mm. These regions are drier than the forest-growing regions of the three countries that have larger eucalypt estates than Australia; i.e. Brazil, India and China. Trees that grow in high-rainfall regions or in riparian habitats are typically the easiest to propagate clonally from cuttings. This has proven true in the development of commercial-scale clonal propagation systems for eucalypts. Successful systems have been established internationally for propagation of cuttings of *E. grandis* (flooded gum), *E. camaldulensis* (river red gum) and *E. deglupta* (rainbow gum), all species from high-rainfall or riparian zones. However, this poses a propagation challenge in subtropical Queensland, where the most suitable species for plantation establishment are adapted to drier conditions. This challenge has been partly addressed by the introduction of *C. torelliana* × *C. citriodora* hybrids, which provide higher potential for clonal propagation than the parental species, *C. citriodora*. Nevertheless, all of the eucalypts used in southern Queensland are difficult to propagate by cuttings. Therefore, in addition to developing capture methods for elite germplasm, this project tested stock plant management and cutting treatment options to improve the clonal propagation potential of some of Queensland's most-promising eucalypt species and hybrids.

One large and comprehensive study assessed the potential for clonal propagation of *C. citriodora*, *E. cloeziana* and *E. dunnii*. The study determined optimal growing conditions for producing cuttings on stock plants, and assessed the effects of the cuttings' nutrient contents on their capacity to form roots successfully. This research was conducted in collaboration with other researchers from the Smart Forests Alliance Queensland and with Dr Mila Bristow from the CRC for Forestry. This allowed a wider range of species to be tested (i.e. including *E. dunnii*) and the full array of plant nutrients to be analysed (N, P, K, Ca, B, Al, Fe, Mg, Mn, Na, S and Zn).

Another large study determined the optimal level of rooting hormone (indole butyric acid; IBA) for propagation of the *Corymbia* hybrid, *C. torelliana* × *C. citriodora* ssp. *variegata*, and the putatively easier-to-propagate *Eucalyptus* hybrid, *E. pellita* × *E. grandis*. This study also assessed the potential use of two novel chemicals, methylcyclopropene (MCP) and aminovinylglycine (AVG), to promote the beneficial effects of IBA on root formation while preventing the detrimental effects of IBA in causing leaf drop. Collaboration with researchers from the Smart Forests Alliance Queensland also allowed the project team to microscopically visualise the sites of root formation in cuttings.

2.2.1 The potential for clonal propagation of Corymbia citriodora, Eucalyptus cloeziana and Eucalyptus dunnii

2.2.1.1 Introduction

Most plantation eucalypts are considered difficult to propagate from cuttings. These include three of the most widely-grown species in subtropical regions, *C. citriodora*, *E. cloeziana* and *E. dunnii*. Many trees that have initially been considered difficult-to-propagate have proven highly amenable to clonal propagation once protocols have been developed that optimise the physiological state of the stock plant, the propagation environment, or post-severance treatments such as auxin application. Commercial nurseries provide partially-protected environments, such as polyethylene chambers or greenhouses with mist irrigation, for cuttings after they have been severed from stock plants. However, climatic control for stock plants is often less than that provided for cuttings.

In this project, we determined the response of *C. citriodora*, *E. cloeziana* and *E. dunnii* stock plants to changing temperature. Specifically, we assessed: (1) the weight and number of cuttings produced by stock plants at four different temperatures; (2) subsequent root formation and cumulative rooted cuttings production when cuttings were treated without or with the auxin, indole-3-butyric acid (IBA); (3) the concentrations of calcium and other nutrients in cuttings at the four temperatures; and (4) relationships between nutrient concentrations and the percentages of cuttings that formed roots. These results will assist in commercial-scale deployment of subtropical eucalypts.

2.2.1.2 Materials and Methods

2.2.1.2.1 Stock plants

Seeds of *C. citriodora* subsp. *variegata* (Woondum State Forest), *E. cloeziana* (Wolvi State Forest) and *E. dunnii* (Koreelah State Forest) were obtained from the Hardwood Tree Improvement Group, Agri-Science Queensland (Gympie). Seeds were sown in January 2009 in potting mix consisting of a 75/25 (v/v) mixture of shredded pine bark and perlite, with 3 kg of 8-9 month slow release Osmocote™ fertiliser (Scotts International, Heerlen, The Netherlands), 3 kg of lime (Unimin, Lilydale, VIC), 1 kg of gypsum (Queensland Organics, Narangba, QLD), 1 kg of Micromax™ granular micronutrients and 1 kg of Hydroflo™ soil wetting agent (both from Scotts Australia, Baulkham Hills, NSW) incorporated per m^3. Seeds were covered with a thin layer of vermiculite and germinated under mist irrigation in a glasshouse in Gympie (26°11'S, 152°40'E). Misting was provided for 10 s every 10 min from 0600 H to 1800 H and for 10s every 20 min from 1800 H to 0600 H.

Seedlings were transplanted in February 2009 into 2.8 L pots filled with the same potting mix (described above), and then transferred randomly into four controlled-temperature glasshouse chambers in Nambour (26°38'S, 152°56'E). The number of seedlings in each chamber was 27, 26 and 27 for *C*. *citriodora*, *E*. *cloeziana* and *E*. *dunnii*, respectively (i.e. 80 seedlings per chamber). Temperatures in all chambers were set at 28°C/23°C (day/night; 0600-1800 H and 1800-0600 H, respectively). Water for initial seedling establishment was provided by trickle irrigation for 1 min every hour from 0800 H to 1700 H, for 1 min at 2200 H, and for 1 min at 0400 H. After 4 weeks, the trickle irrigation was reduced to 1 min every 3 hours from 0800 H to 1700 H, 1 min at 2200 H, and 1 min at 0400 H.

Commencing in April 2009, seedlings were managed as stock plants by pruning at 3-week intervals to a height of ~30 cm and a canopy diameter of ~20 cm. The last pruning before imposition of the experimental treatments was performed on 8 June 2009. Irradiance in the stock plant chambers was monitored on four cloudless days between April and August 2009 (Fig. 2.9a) using a quantum sensor (LICOR LI-250A, Lincoln, NE).

2.2.1.2.2 Experimental design

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

All available cuttings of all stock plants were harvested at 2, 5, 8, 11 and 14 weeks after commencement of the four temperature treatments. The total fresh weight and number of cuttings was recorded for each stock plant on each occasion. A random sample of nine cuttings per stock plant (or all cuttings, if less than nine were available) was then prepared for setting on each occasion by trimming cuttings to approximately 5-cm length and pruning half to two-thirds of the length of each leaf. Cuttings of *C. citriodora* possessed a single leaf because leaves were alternate, whereas cuttings of *E. cloeziana* and *E. dunnii* possessed two leaves because leaves were opposite, in juvenile-phase shoots.

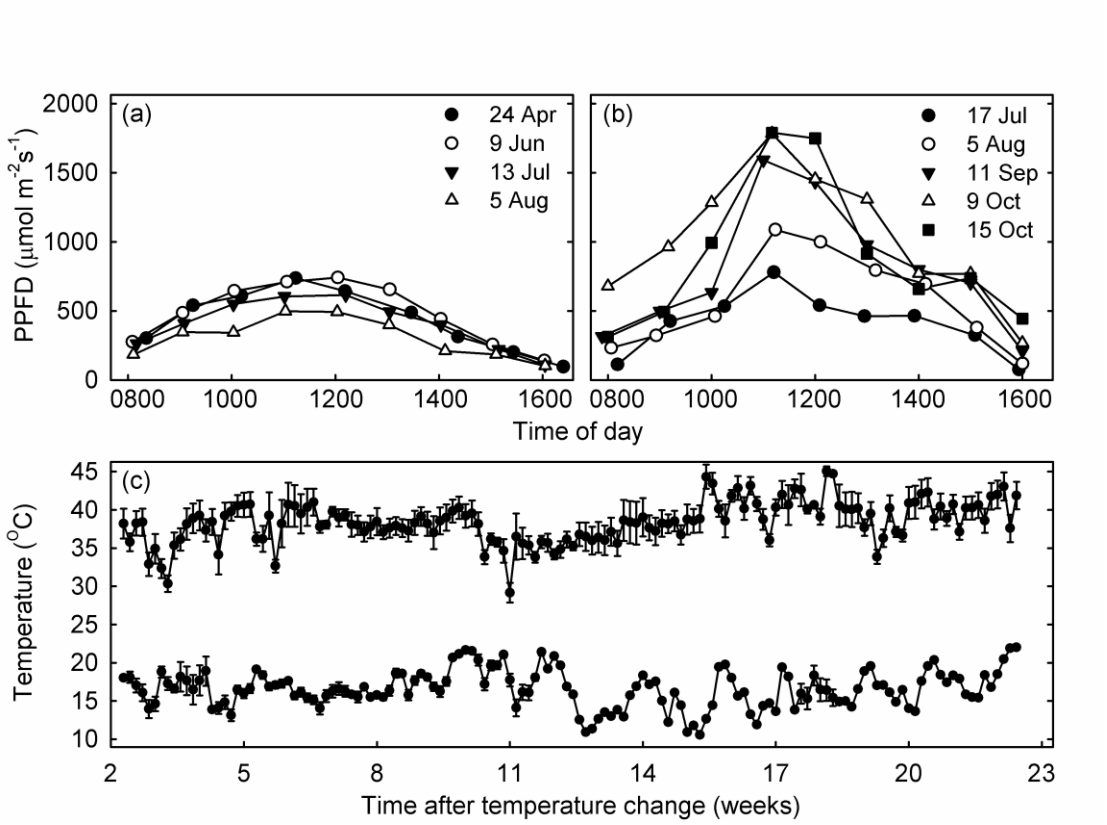

Fig. 2.9a. Photosynthetic photon flux densities (PPFD) in the (a) stock plant chamber and (b) glasshouse, and (c) maximum and minimum temperatures (± s.e.) in the glasshouse, during propagation of *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* cuttings

All nine cuttings from a stock plant were allocated randomly to one of the two hormone treatments, 0 or 3 g indole-3-butyric acid (IBA)/kg talcum powder, derived from a previous study of adventitious root formation in *C. citriodora* cuttings. Cuttings were dipped 0.5-cm into treatment powder for about 1 s and placed 1 cm deep in a 12-cm^3 tube containing a 75/25(v/v) mixture of perlite and shredded pine bark with 3 kg of 8-9 month slow-release Osmocote^{TM} fertiliser and 1 kg of gypsum incorporated per m^3. Trays were placed in the same glasshouse where the seeds had been germinated (see above), with mist irrigation provided for 10 s every 15 min from 0600H to 1800H and for 10 s every 20 min from 1800H to 0600H. Trays were placed on TPS080 heated root-beds (Thermofilm, Springvale, VIC) to maintain ~25°C at the base of each tray for the first 4 weeks, and then moved to ambient glasshouse conditions (Figs 2.9b, c) for a further 5 weeks. Temperatures were recorded in the glasshouse for the duration of the experiment using Tinytalk data loggers (RS Components, Smithfield, NSW) and irradiance was measured on five cloudless days using a quantum sensor (LICOR LI-250A, Lincoln, NE). Cuttings were gently removed from the propagation mix after 9 weeks, and the proportion of cuttings forming roots was recorded for each stock plant.

2.2.1.2.3 Nutrient analyses

All available cuttings of three stock plants per species from each temperature were harvested at 2, 8 and 14 weeks after commencement of the four temperature treatments. Different stock plants were harvested on each occasion. The cuttings were placed in a paper bag, dried for 7 d at 65°C, weighed, and then ground using a Retsch MM200 tissue homogeniser (Retsch, Haan, Germany). The concentrations of N and S were determined by combustion analysis 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 after nitric and perchloric acid digestion.

2.2.1.2.4 Statistical analyses

Cumulative fresh weight and number of cuttings per stock plant were analysed by 1-way ANOVA, comparing four temperature treatments within each species. Proportions of cuttings forming roots and cumulative number of rooted cuttings per stock plant were analysed by 1-way ANOVA, comparing four temperatures within each species and IBA level, because significant temperature × IBA interactions were detected by 2-way ANOVA. Differences between IBA levels within each temperature were compared using t-tests. Nutrient concentrations were also analysed by 1-way ANOVA, comparing four temperatures within each harvest date or comparing three harvest dates within each temperature, because significant temperature × harvest date interactions were detected by 2-way ANOVA. Post-hoc least significant difference (LSD) tests were performed only when significant differences were detected by ANOVA. Fresh weight or number data were square root or log transformed, and proportions were arcsine square root transformed, when variance was heterogeneous. In addition, linear regressions using mean nutrient concentration and rooting percentage as the independent and dependent variables, respectively, were calculated for each species. Means are reported with standard errors, and treatment differences or interactions were regarded as significant at $P < 0.05$.

2.2.1.3 Results

Stock plants of all three species produced the highest weight and highest number of cuttings when they were grown at 33°C or 28°C (Fig. 2.10). The means for these two temperatures did not differ significantly, except that *E. cloeziana* stock plants produced more cuttings at 33°C than at 28°C (Fig. 2.10D). The lowest weights of cuttings were obtained at 18°C for *C. citriodora* and *E. dunnii* (Figs 2.10A, 2.10E) and at 18°C and 23°C for *E. cloeziana* (Fig. 2.10C). The lowest numbers of cuttings were obtained at 18°C and 23°C in all three species (Figs 2.10B, 2.10D, 2.10F). The final numbers of cuttings per stock plant ranged from 53.1 –

139.5 for *C. citriodora*, 35.1 – 102.1 for *E. cloeziana* and 93.0 – 196.5 for *E. dunnii*, depending on the temperature.

The percentage of cuttings that formed roots was low for all three species and, on most occasions, stock plant temperature had no significant effect on rooting (Fig. 2.11). However, cuttings from stock plants grown at 33°C provided higher rooting than cuttings from some or all other treatments on one occasion for *E. cloeziana* (Fig. 2.11C) and on three occasions for *E. dunnii* (Figs 2.11E, 2.11F). When rooting percentages for each stock plant were averaged across the five settings, stock plant temperature was found to affect rooting in all species (Figs 2.11A, 2.11C, 2.11D, 2.11E, 2.11F) with the exception of *C. citriodora* cuttings that were treated with IBA (Fig. 2.11B). Stock plants grown at 33°C consistently provided one of the highest average rooting percentages, although differences among temperatures varied depending on the species and the IBA treatment. IBA significantly increased the rooting percentage on just one occasion for each species (Figs 2.11B, 2.11D, 2.11F). Average rooting percentages (i.e. averaged across all five settings) were only increased significantly by IBA when *E. cloeziana* was grown at 28°C (Fig. 2.11D) or when *E. dunnii* was grown at 33°C (Fig. 2.11F). Average rooting percentages ranged from 1.1 – 14.9 % for *C. citriodora*, 1.4 – 13.7 % for *E. cloeziana* and 1.8 – 21.7 % for *E. dunnii*, depending on the stock plant temperature and IBA treatment.

Final production of rooted cuttings was highest for *C. citriodora* when stock plants were grown at 28°C or 33°C, regardless of the IBA treatment (Figs 2.12A, 2.12B). For *E. cloeziana*, a stock plant temperature of 33°C provided the highest number of rooted cuttings if the cuttings were not treated with IBA (Fig. 2.12C); however, stock plant temperature had no significant effect if the cuttings were treated with IBA (Fig. 2.12D). For *E. dunnii*, stock plant temperatures between 23°C and 33°C provided the highest rooted cuttings production when the cuttings were not treated with IBA (Fig. 2.12E), whereas 33°C was the optimal temperature if the cuttings were treated with IBA (Fig. 2.12F). Application of IBA significantly increased rooted cuttings production from *E. dunnii* stock plants grown at 33°C, but it had no significant effect on rooted cuttings production of *E. dunnii* following the other three temperatures (18, 23 and 28°C), or at any temperature for the other two species (*C. citriodora* and *E. cloeziana*). Final numbers of rooted cuttings per stock plant ranged from 1.1 – 24.9 for *C. citriodora*, 1.0 – 11.8 for *E. cloeziana* and 1.5 – 51.6 for *E. dunnii*, depending on the temperature and IBA treatments.

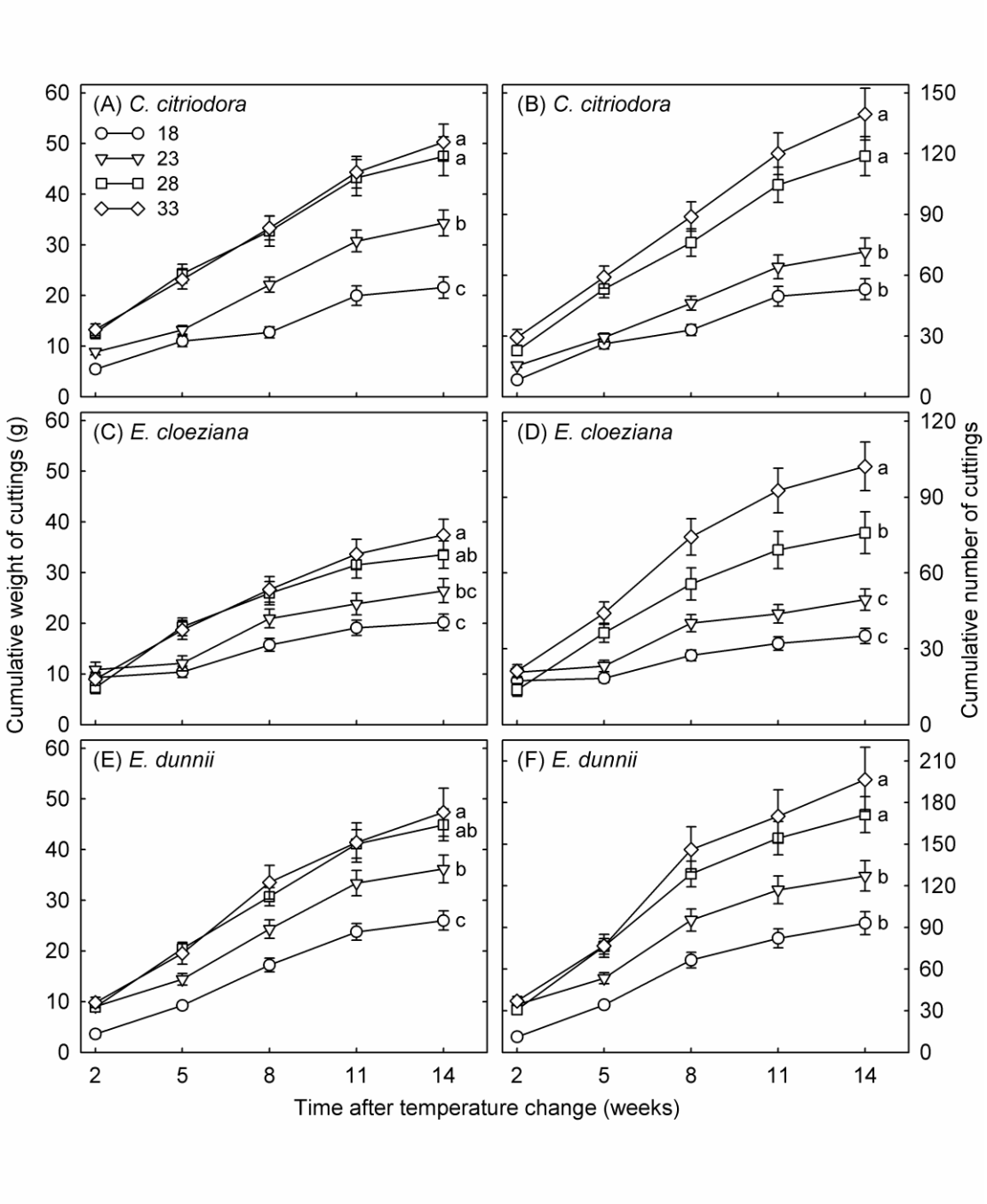

Fig. 2.10. (A, C, E) Cumulative weight and (B, D, F) cumulative number of cuttings produced per *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* stock plant after temperature was changed from 28°C to 18, 23, 28 or 33°C. Final means (± s.e.) with different letters are significantly different (ANOVA and LSD test, $P < 0.05$, n = 18–24)

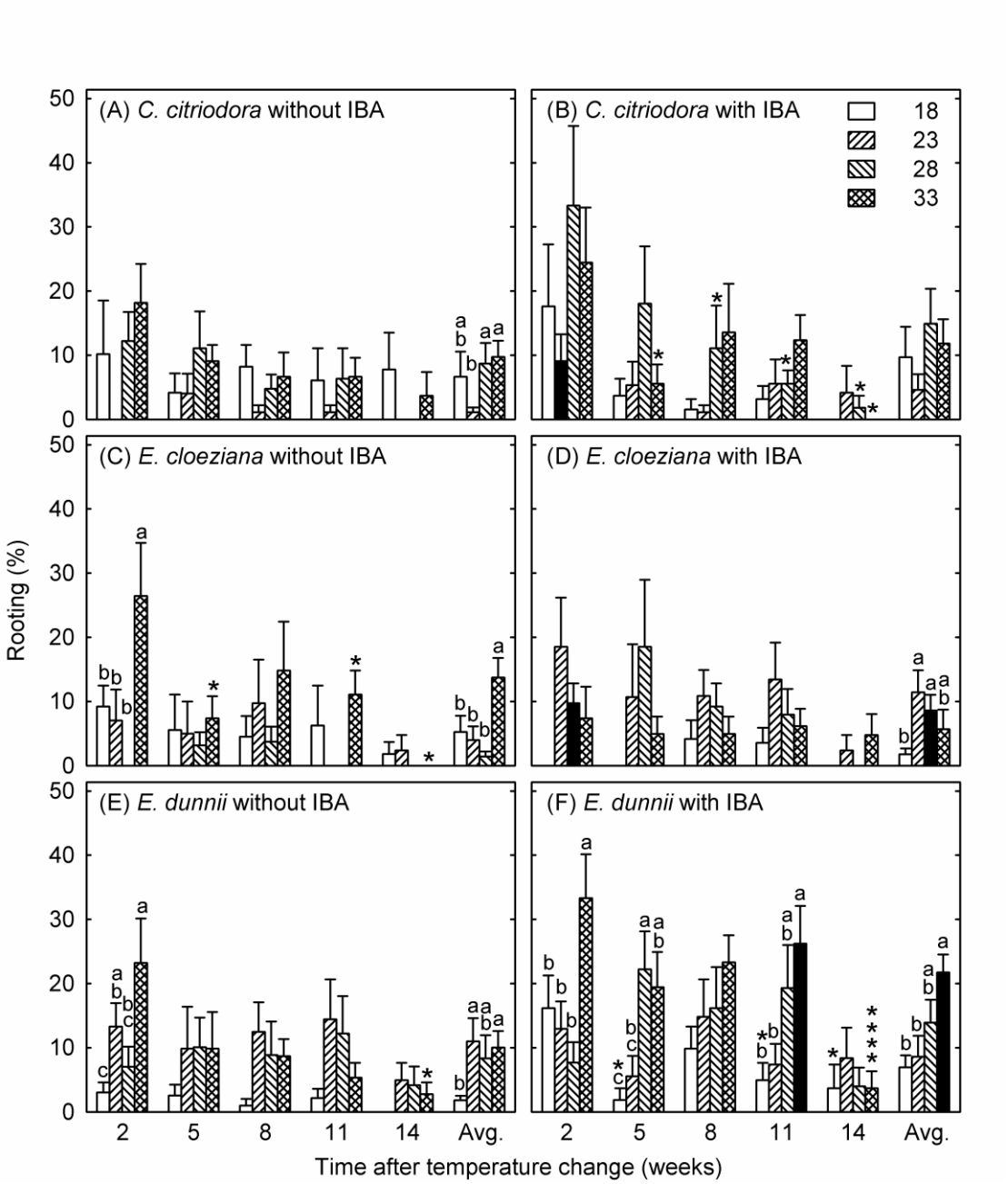

Fig. 2.11. Percentage of cuttings that formed adventitious roots (A, C, E) without indole-3-butyric acid (IBA) or (B, D, F) with IBA after temperature of *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* stock plants was changed from 28°C to 18, 23, 28 or 33°C. 'Avg' refers to the average rooting percentage across the five harvests. Temperature treatment means (+ s.e.) with different letters are significantly different; asterisks (*) indicates a significant decline from the first harvest; black bars indicate a significant IBA effect (ANOVA and LSD test, or t-test; $P < 0.05$, n = 9–12)

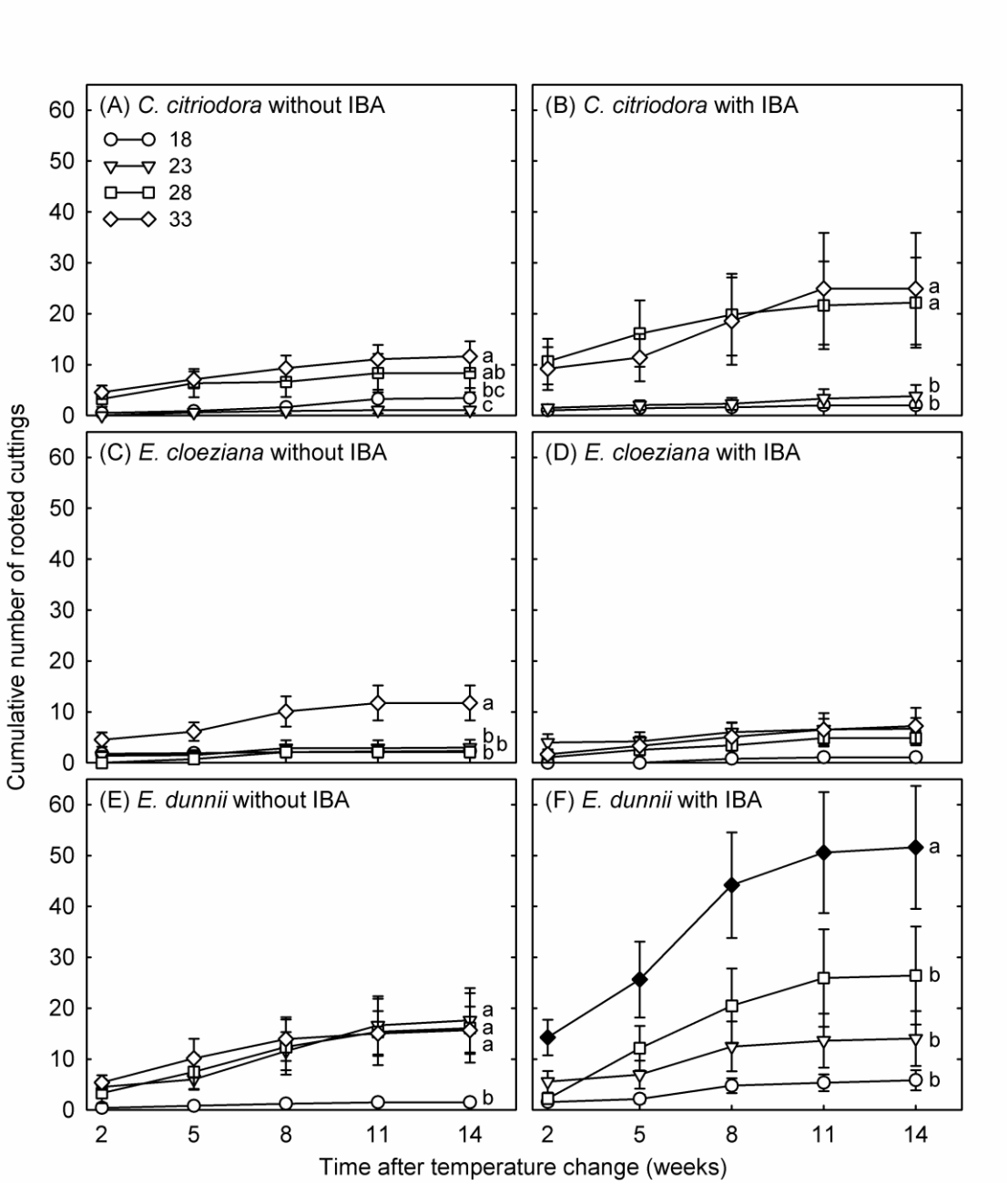

Fig. 2.12. Cumulative number of rooted cuttings produced per stock plant after temperature of *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* stock plants was changed from 28°C to 18, 23, 28 or 33°C, and cuttings were treated (A, C, E) without indole-3-butyric acid (IBA) or (B, D, F) with IBA. Final means (± s.e.) with different letters are significantly different; black symbols indicate a significant effect of IBA on final number of rooted cuttings (ANOVA and LSD test, or t-test; $P < 0.05$, n = 9–12)

Stock plant temperature generally did not affect the nutrient concentrations of cuttings, and so data for N, P, K, Al, Fe, Mg, Mn, Na, S and Zn concentration are not presented. Nutrient concentrations sometimes declined between 2 and 8, or between 2 and 14, weeks after imposition of the varying stock plant temperatures; however, the numbers of significant declines in nutrient concentration were only 2 out of the 48 potential cases (12 nutrients × 4 temperatures) for *E. cloeziana* (N, P at 33°C) and 5 of the 48 cases for *E. dunnii* (N, P, K, S at 23°C; B at 33°C), but 14 of the 48 cases for *C. citriodora* (P, K, B at 18°C; P, K, Fe at 23°C; N, P, K, B, Fe at 28°C; N, K, Fe at 33°C).

Stock plant temperature had no significant effect on Ca concentration of cuttings (Fig. 2.13), and the percentage of cuttings that formed roots was related to Ca concentration only when *E. dunnii* cuttings were treated with IBA (Fig. 2.13F). Rooting percentages were generally not related to concentrations of other nutrients (Table 2.5), with the exception of B (Fig. 2.14). Stock plant temperature had little or no effect on B concentration (Figs 2.14A, 2.14C, 2.14E), and B concentration only declined significantly by the final harvest date in 3 out of 12 possible instances (Figs 2.14A, 2.14E). However, rooting percentages were positively related to B concentration in *C. citriodora* (Fig. 2.14B; Table 2.5) and *E. dunnii* (Fig. 2.14F; Table 2.5) regardless of IBA treatment, and in *E. cloeziana* (Fig. 2.14D; Table 2.5) when the cuttings were not treated with IBA.

2.2.1.4 Discussion: The potential for clonal propagation of Corymbia citriodora, Eucalyptus cloeziana and Eucalyptus dunnii

Low temperatures greatly reduced the number of cuttings produced by *C. citriodora*, *E. cloeziana* and *E. dunnii* stock plants but they did not reduce the Ca concentration of cuttings and they had variable effects on the ensuing percentage of cuttings that formed roots. Cuttings of all species proved difficult-to-root under winter and spring conditions despite the use of root-bed heating for the first 4 weeks after severance from the stock plant. The optimal temperatures for shoot production by *C. citriodora*, *E. cloeziana* and *E. dunnii* stock plants were 28 and 33°C, consistent with previous reports of the optimal temperatures for photosynthesis in *E. argophloia* and *E. cloeziana* seedlings. The final numbers of rooted cuttings produced by *C. citriodora* at the different stock plant temperatures primarily reflected the number of cuttings produced by the stock plants rather than their subsequent rooting percentages, because low stock plant temperatures greatly affected shoot production but usually did not affect adventitious root production. However, final production of *E. cloeziana* and *E. dunnii* rooted cuttings in the current study was related to both the number of cuttings produced by stock plants and their rooting percentages, because stock plant temperatures greatly affected both shoot production and subsequent adventitious root production.

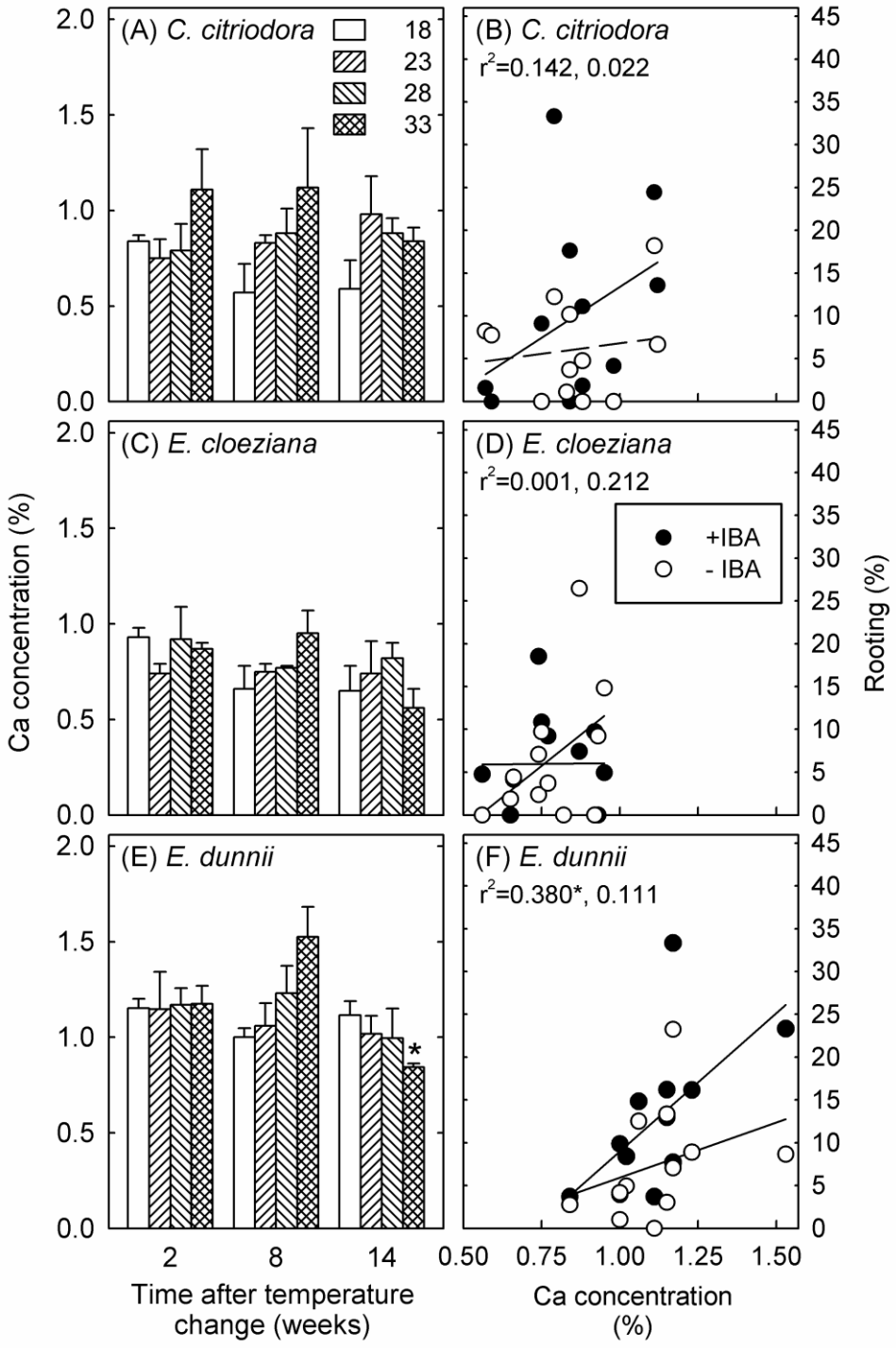

Fig. 2.13. (A, C, E) Ca concentration of cuttings, and (B, D, F) regressions between the percentage of cuttings that formed roots and Ca concentration, after temperature of *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* stock plants was changed from 28°C to 18, 23, 28 or 33°C. Two r^2-values refer to cuttings treated with or without indole-3-butyric acid (IBA), respectively. Ca concentration (+ s.e.) did not vary significantly among temperatures or harvests (ANOVA; *P* > 0.05, n = 3); a significant regression is indicated by an asterisk (*) (*P* < 0.05, n = 12)

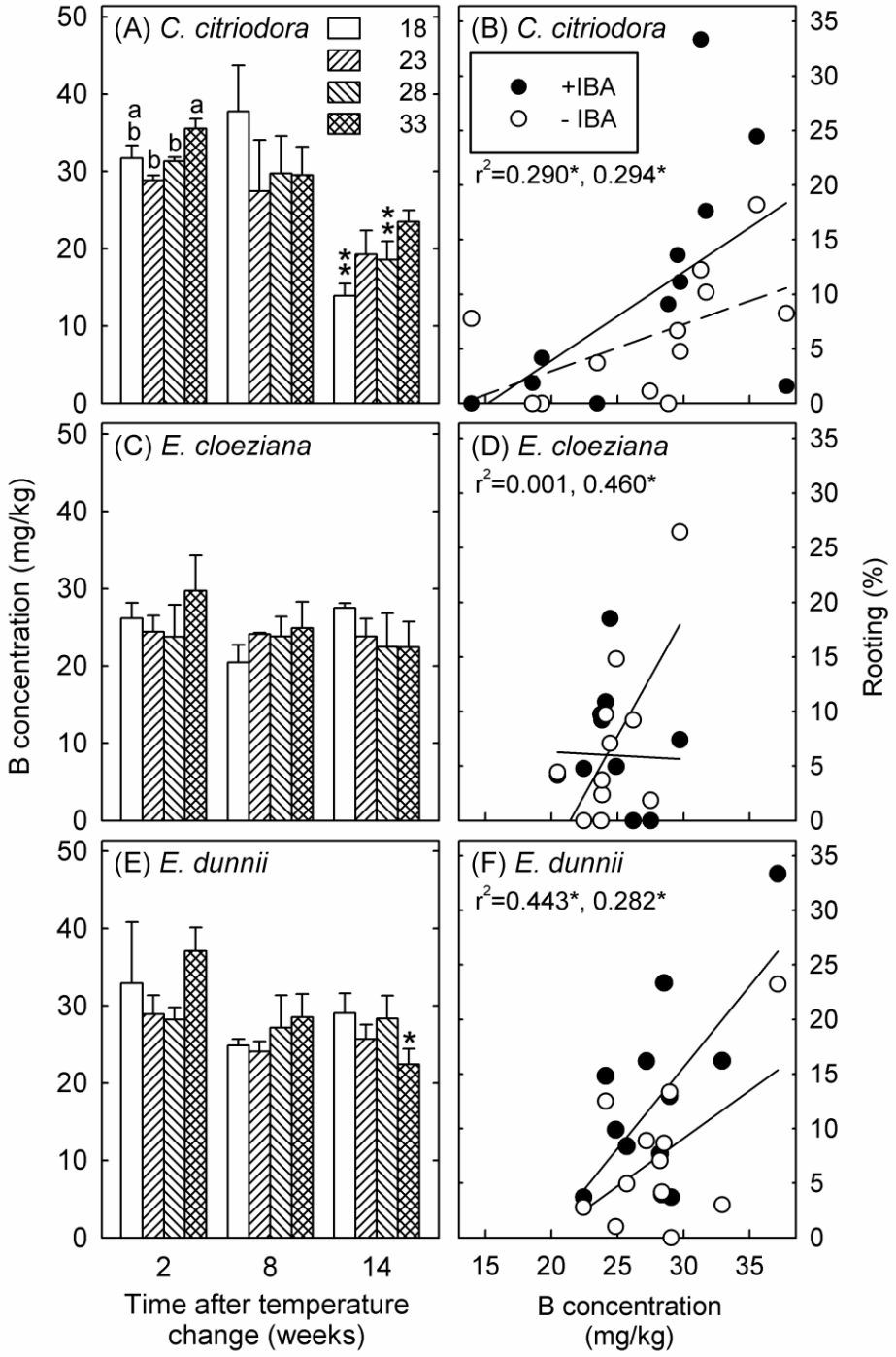

Fig. 2.14. (A, C, E) B concentration of cuttings, and (B, D, F) regressions between the percentage of cuttings that formed roots and B concentration, after temperature of *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* stock plants was changed from 28°C to 18, 23, 28 or 33°C. Two r^2-values refer to cuttings treated with or without indole-3-butyric acid (IBA), respectively. B concentrations (+ s.e.) with different letters are significantly different and B concentrations marked with an asterisk (*) are significantly lower than at the first harvest (ANOVA and LSD test, $P < 0.05$, n = 3); significant regressions are also indicated by an asterisk ($P < 0.05$, n = 12)

Table 2.5. Coefficients of determination (r^2) for linear regressions between percentage of cuttings forming roots and nutrient concentration in *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* treated without or with indole-3-butyric acid (IBA)

Nutrient	*C. citriodora*		*E. cloeziana*		*E. dunnii*	
	- IBA	+ IBA	- IBA	+ IBA	- IBA	+ IBA
N	0.181	0112	**0.293***	**0.257**	0.038	<0.001
P	**0.404***	**0.431***	**0.255***	0.012	0.016	0.012
K	**0.523***	**0.550***	**0.391***	0.232	0.215	0.131
Ca	0.022	0.142	0.212	<0.001	0.111	**0.380***
B	**0.294***	**0.290***	**0.460****	0.001	**0.282***	**0.443***
Al	0.027	0.016	0.035	<0.001	**0.266***	**0.508***
Fe	0.155	0.045	0.033	0.014	0.199	0.228
Mg	0.194	**0.248***	<0.001	0.094	0.064	0.013
Mn	0.030	0.133	0.120	0.036	0.008	0.056
Na	**0.481***	**0.386***	0.028	0.118	0.037	0.019
S	0.077	0.162	0.149	0.107	**0.318***	0.240
Zn	0.011	0.051	0.056	0.001	0.070	0.033

Coefficients indicated in bold with an asterisk (*) are significant (linear regression; $P < 0.05$; n = 12)

Low stock plant temperatures did not reduce the Ca concentration of *C. citriodora*, *E. cloeziana* or *E. dunnii* cuttings, and the concentrations of other nutrients were not, or were only slightly, affected by temperature. The concentrations of some nutrients such as N, P, K, B and Fe occasionally declined by the end of the experiment, as did rooting percentages. The effects of individual nutrients on rooting cannot be clearly delineated because individual nutrient concentrations were often correlated with each other. Nonetheless, rooting percentages were related in all species to the concentration of only one nutrient, B. The relationships between rooting and B concentration in *C. citriodora*, *E. cloeziana* and *E. dunnii* are very similar to that found recently in Brazil from a clone of *E. grandis* × *E. urophylla*, in which rooting percentage was related positively to the concentration of B in cuttings but not to the concentrations of N, P, K, Ca, Mg, S, Zn, Fe or Mn. Few studies have attempted to characterize specific effects of B on adventitious root formation in woody plants and so further research is warranted to assess the effects of B concentrations, independently of other nutrients, on adventitious root formation in cuttings of *C. citriodora*, *E. cloeziana*, *E. dunnii* and other eucalypts.

Application of IBA at 3 g/kg did not increase the average percentage of *C. citriodora* cuttings that formed roots, as found previously for *C. citriodora* using 0, 1, 3 and 8 g/kg IBA. In

contrast with *C. citriodora*, IBA raised the average percentage of *E. cloeziana* and *E. dunnii* cuttings that formed roots in the present study, though only when cuttings were taken from stock plants grown at one of the optimal temperatures for shoot and adventitious root production, 28°C (for *E. cloeziana*) or 33°C (for *E. dunnii*). This increased rooting following IBA application accords with results from other *Eucalyptus* species and hybrids, including *E. benthamii* × *E. dunnii*, *E. grandis*, *E. grandis* × *E. urophylla*, *E. globulus* and *E. pellita* × *E. tereticornis*.

2.2.1.5 Conclusion: The potential for clonal propagation of Corymbia citriodora, Eucalyptus cloeziana and Eucalyptus dunnii

We recommend that stock plants of *Corymbia citriodora*, *Eucalyptus cloeziana* and *Eucalyptus dunnii* be grown at warm temperatures (28–33°C) for as long as possible each day to increase the production of rooted cuttings.

Stock plant temperature regulated the production of rooted cuttings in all three species, firstly by regulating the number of shoots produced by stock plants and, secondly, by affecting the ensuing percentage of cuttings that formed adventitious roots. Shoot production was the primary mechanism by which stock plant temperature affected rooted cutting production in *C. citriodora*, but both shoot production and adventitious root production were key determinants of rooted cutting production between the different stock plant temperatures in *E. cloeziana* and *E. dunnii*. The effects of lower stock plant temperatures on rooting were not the result of reduced Ca concentrations in cuttings, but relationships were found between adventitious root formation and B concentration in all three species. Stock plants of *C. citriodora*, *E. cloeziana* and *E. dunnii* could produce, on average, as many as 25, 12 and 52 rooted cuttings, respectively, over a 14-week collection period, sufficient to maintain rooted cuttings in a nursery clonal archive and establish clonal field tests. However, further research is required to optimise the propagation techniques for plantation tree production because average rooting percentages (up to 15% for *C. citriodora*, 14% for *E. cloeziana* and 22% for *E. dunnii*) remain far below the 70%-level preferred by commercial nurseries.

2.2.2 The optimal level of rooting hormone for propagation of the hybrids, *Corymbia torelliana* × *Corymbia citriodora* and *Eucalyptus pellita* × *Eucalyptus grandis*

2.2.2.1 Introduction

Application of auxin, in particular indole-3-butyric acid (IBA), is one of the most common treatments to enhance rooting of cuttings. IBA is used on a wide range of tree species including eucalypts 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. However, some species and hybrids appear unresponsive to auxin and high doses can cause cutting death. High IBA doses have previously reduced rooting and increased defoliation and death of *C. citriodora*, *C. henryi*, *C. torelliana* and *C. citriodora* × *C. torelliana* cuttings. The mechanism of IBA-induced defoliation and death remains unknown.

Leaf senescence and abscission are generally caused by accumulation of another hormone, ethylene. Auxin application commonly stimulates ethylene production, and so auxin-induced ethylene accumulation may be the primary cause of defoliation in eucalypt cuttings. The ethylene perception inhibitor, 1-methylcyclopropene (MCP), and synthesis inhibitor, aminoethoxyvinylglycine (AVG), are used commercially to inhibit ethylene responses and prevent or delay fruit ripening, fruit abscission or leaf abscission. Neither ethylene inhibitor has been tested on woody plant cuttings. However, incorporating the ethylene perception inhibitor, silver thiosulphate (STS), into IBA-containing medium has induced root formation in micropropagated shoots of *Corymbia maculata*, indicating that ethylene generated in the presence of IBA prevents adventitious root induction. Defoliation and death of eucalypt cuttings may be the result of IBA-induced ethylene production, in which case the deleterious effects of high IBA doses could be prevented by ethylene inhibitors such as MCP, AVG or STS. The timing of root initiation may be critical in determining the optimal application time for ethylene inhibitors because some ethylene can be beneficial during the initiation phase of root formation. The precise timing of root initiation requires microscopic examination, and there have been no previous studies on the precise timing of root initiation in eucalypt cuttings.

The objective of this study was to develop an improved technique for propagation of cuttings of *C. torelliana* × *C. citriodora* and *E. pellita* × *E. grandis*. Specifically, the aims were to determine: (1) the relationships between IBA dose, the percentage of cuttings that form roots, and the number of adventitious roots per rooted cutting; (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 is 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.

2.2.2.2 Materials and Methods

2.2.2.2.1 Stock plants

Seeds were obtained from the Hardwood Tree Improvement Group, Agri-Science Queensland, Gympie. 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 in the vicinity of 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 in Oct 2009, and germinated under mist irrigation (10 s every 10 min from 0600–1800 H and 10 s every 20 min from 1800–0600 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^{TM} fertiliser (Scotts International, Heerlen, The Netherlands), 3 kg lime (Unimin, Lilydale, VIC), 1 kg Micromax^{R} micronutrients (Scotts Australia, Baulkham Hills, NSW), 1 kg Hydroflow^{TM} wetting agent (Scotts Australia, Baulkham Hills, NSW) 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 s every 30 min from 0600–1800 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-cm and 50-cm height by regular pruning. Each stock plant was provided with 150 mL of foliar fertiliser, containing 18 g/L Flowfeed GF9 (Growforce, Acacia Ridge, QLD), 3 mL/L Firmrite (Spraygro Liquid Fertilizers, Gillman, SA) and 500 mg/L $MgSO_4$, 7 d prior to each harvest of cuttings.

2.2.2.2.2 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. Ten cuttings per hybrid on each occasion were selected randomly, placed in separate paper bags, dried for 7 days at 55°C, and weighed.

Another 1350 cuttings from each hybrid on each occasion were allocated randomly to nine treatments in each of the respective experiments (1 and 2; see 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. The remaining 10 tubes in each tray were filled with cuttings of *E. argophloia*, collected from stock plants derived from 11 open-pollinated seedlots from the Agri-Science Queensland seed orchard at Dunmore (27°34′S, 151°05′E). Rooting of this species was less than 3% across all treatments and so *E. argophloia* data is not presented.

Each cutting was dipped 0.5-cm into treatment powder for 1 s 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^{TM} fertiliser and 1 kg of gypsum incorporated per m^3. 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 IBA, and cuttings in the remaining three treatments were dipped in powder containing 8 g/kg IBA. The MCP or AVG component of each of the nine treatment combinations was applied subsequently (see Experiments 1 and 2, below).

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 misting at the University of the Sunshine Coast was provided for 60 s every 5 min (day and night), whereas heavier misting at Gympie was provided for 10 s every 10 min (0600–1800 H) and 10 s every 20 min (1800–0600 H). Temperatures were recorded for the duration of experiments using Tinytalk dataloggers (RS Components, Smithfield, NSW). 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).

2.2.2.2.3 Experiment 1: MCP application

The 30 trays within each of the three IBA concentrations (0, 3 or 8 g/kg) were separated randomly 8 d after setting into three treatments, each with ten trays to be treated with one of three MCP concentrations: 0, 400 or 800 nL/L. This timing (8 d after setting) was determined from preliminary experiments, which found no significant effects of time of application (0, 4 or 8 d) of MCP or AVG on root formation or cutting growth. Trays were placed into 80-L plastic tubs immediately 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 (1700 – 0700 H), the tubs were then opened, and the trays were returned to the propagation chamber.

2.2.2.2.4 Experiment 2: AVG application

The 30 trays within each IBA concentration were separated randomly 8 d after setting into their three treatments, each with ten trays to be treated with one of three AVG concentrations: 0, 125 or 250 mg/ L. The trays were placed into 80-L plastic tubs immediately 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 (1700 – 0700 H) to allow AVG uptake before the cuttings were returned to the glasshouse.

2.2.2.2.5 Defoliation, death, and root and shoot development

The numbers of defoliated cuttings and dead cuttings in each replicate were recorded every 7 d from 0–56 d 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 d 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. Rooted cuttings were rinsed carefully to remove the propagation mixture, and the roots were excised from the shoot. The roots and shoot were placed in separate paper bags, dried for 7 days at 55°C, and weighed.

2.2.2.2.6 Timing of adventitious root formation

An additional 30 cuttings of each hybrid from the first occasion (1 Mar 2010) were dipped in talcum powder containing either no IBA (15 cuttings) or 8 g/kg IBA (15 cuttings). The method was exactly as described under 'General methods' (above) and the two trays of cuttings were placed alongside those of Experiment 1 (above). Five *C. torelliana* × *C. citriodora* and five *E. pellita* × *E. grandis* cuttings from each treatment were sampled randomly at 7 d, 14 d and 21 d 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 later 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, QLD), dewaxed with xylene and ethylene, stained with safranin and fast green, and mounted with Permount mounting medium (ProSciTech, Thuringowa, QLD). All sections were examined for adventitious root formation using an Eclipse E200 microscope (Nikon, Lidcombe, NSW).

2.2.2.2.7 Statistical analyses

The final proportions of defoliated cuttings, dead cuttings, and cuttings with roots, as well as adventitious root number, root weight, shoot weight and total weight of cuttings, were analysed by 1-way ANOVA for each hybrid. The use of 1-way rather than 2-way ANOVA allowed comparison of each treatment with the control (i.e. untreated cuttings) and all other treatments. Analyses of defoliated cuttings, dead cuttings, and cuttings with roots included all cuttings, but analyses of adventitious root number, root weight, shoot weight and total weight included only the rooted cuttings. Proportions were arcsine square root transformed, and root number or weights were square root transformed, when variance was heterogeneous. Duncan's multiple range tests were performed when significant differences were detected by ANOVA. Means are reported with standard errors and treatment differences were regarded as significant at $P < 0.05$.

2.2.2.3 Results

2.2.2.3.1 Experiment 1: MCP application

IBA and MCP treatments did not significantly affect the percentage of cuttings with abscised leaves or the percentage of dead cuttings for either hybrid (Fig. 2.15). 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. 2.15c) and between 3 and 5 weeks after setting for *E. pellita* × *E. grandis* (Fig. 2.15d). Mortality was highest in the eighth week after setting for both hybrids (Figs 2.15e, f). The final levels of leaf abscission did not vary significantly among treatments for *C. torelliana* × *C. citriodora* (36.8 ± 4.6% – 50.1 ± 4.7%) (Fig. 2.15c) or *E. pellita* × *E. grandis* (25.3 ± 4.1% – 30.6 ± 3.2%) (Fig. 2.15d). Cutting death also did not vary significantly among treatments for *C. torelliana* × *C. citriodora* (14.7 ± 3.7% – 26.2 ± 3.6%) (Fig. 2.15e) or *E. pellita* × *E. grandis* (6.0 ± 1.8% – 16.6 ± 4.6%) (Fig. 2.15f).

Some of the combined IBA/MCP treatments increased the percentage of cuttings that produced roots compared with untreated cuttings. The percentage of *C. torelliana* × *C. citriodora* cuttings that formed roots was increased when the cuttings were treated with 3 g/kg IBA and 400 or 800 nL/L MCP, or with 8 g/kg IBA and 400 nL/L MCP (Fig. 2.16a). The percentages of *C. torelliana* × *C. citriodora* cuttings that formed roots following these treatments were 36.1 ± 6.0% – 42.1 ± 3.9% whereas only 20.8 ± 2.3% of untreated cuttings formed roots. 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. 2.16a). The treatments, 3 g/kg IBA combined with 400 or 800 nL/L MCP, and all combinations of 8 g/kg IBA with or without MCP, significantly increased the percentage of *E. pellita* × *E. grandis* cuttings with roots (56.3 ± 4.2% – 58.7 ± 5.5%) compared with untreated cuttings (39.3 ± 5.5%) (Fig. 2.16b).

Some of the combined IBA/MCP treatments significantly increased the number of adventitious roots per rooted cutting. The treatments, 8 g/kg IBA combined with 400 or 800 nL/L MCP, significantly increased the number of adventitious roots from *C. torelliana* × *C. citriodora* cuttings (Fig. 2.16c). The numbers of adventitious roots per rooted cutting following these treatments were 4.2 ± 0.4 and 3.2 ± 0.4 roots, respectively, compared with 2.0 ± 0.3 roots per untreated cutting. The number of roots per rooted *E. pellita* × *E. grandis* cutting was significantly higher when the cuttings were treated with 3 g/kg IBA and 800 nL/L MCP or with 8 g/kg IBA with or without MCP (2.9 ± 0.3 – 4.2 ± 0.5 roots) than when the cuttings were untreated (1.9 ± 0.2 roots) (Fig. 2.16d). In addition, significantly more adventitious roots were formed on *E. pellita* × *E. grandis* cuttings when the cuttings were treated with 8 g/kg IBA (4.2 ± 0.5 roots) than when they were treated with 3 g/kg IBA (2.7 ± 0.3 roots) (Fig. 2.16d).

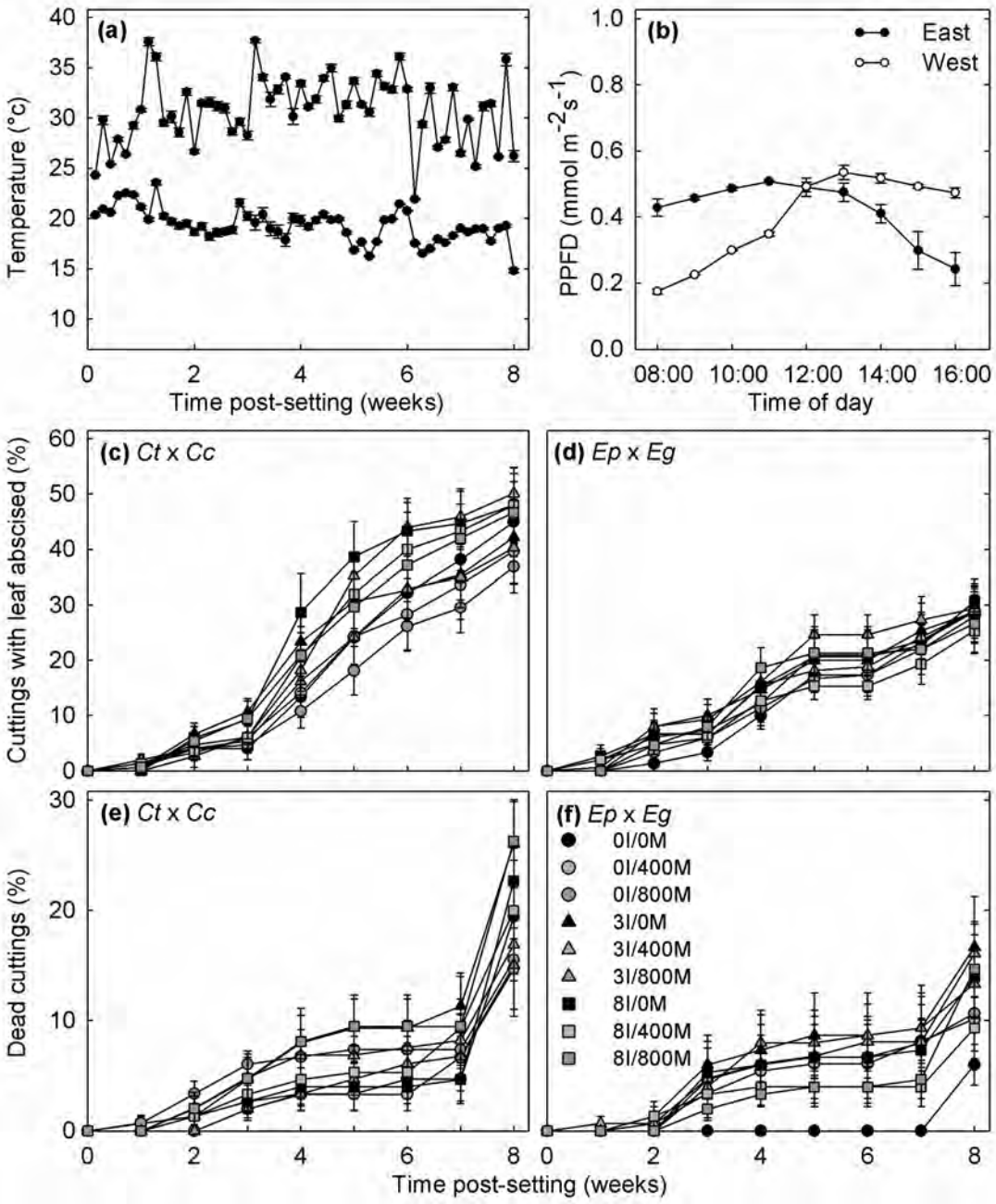

Fig. 2.15. Experiment 1: Daily maximum and minimum temperatures (a) and photosynthetic photon flux densities (PPFD) (b) in the propagation chamber, and percentage of cuttings with the leaf abscised (c, d) and mortality (e, f) for *Corymbia torelliana* × *C. citriodora* (*Ct* × *Cc*) and *Eucalyptus pellita* × *E. grandis* (*Ep* × *Eg*) cuttings subjected to one of nine combinations of IBA (I) (0, 3 or 8 g/kg) and MCP (M) (0, 400 or 800 nL/L). Final means (+ s.e.) within a hybrid do not differ significantly (ANOVA, *P* > 0.05, n = 10)

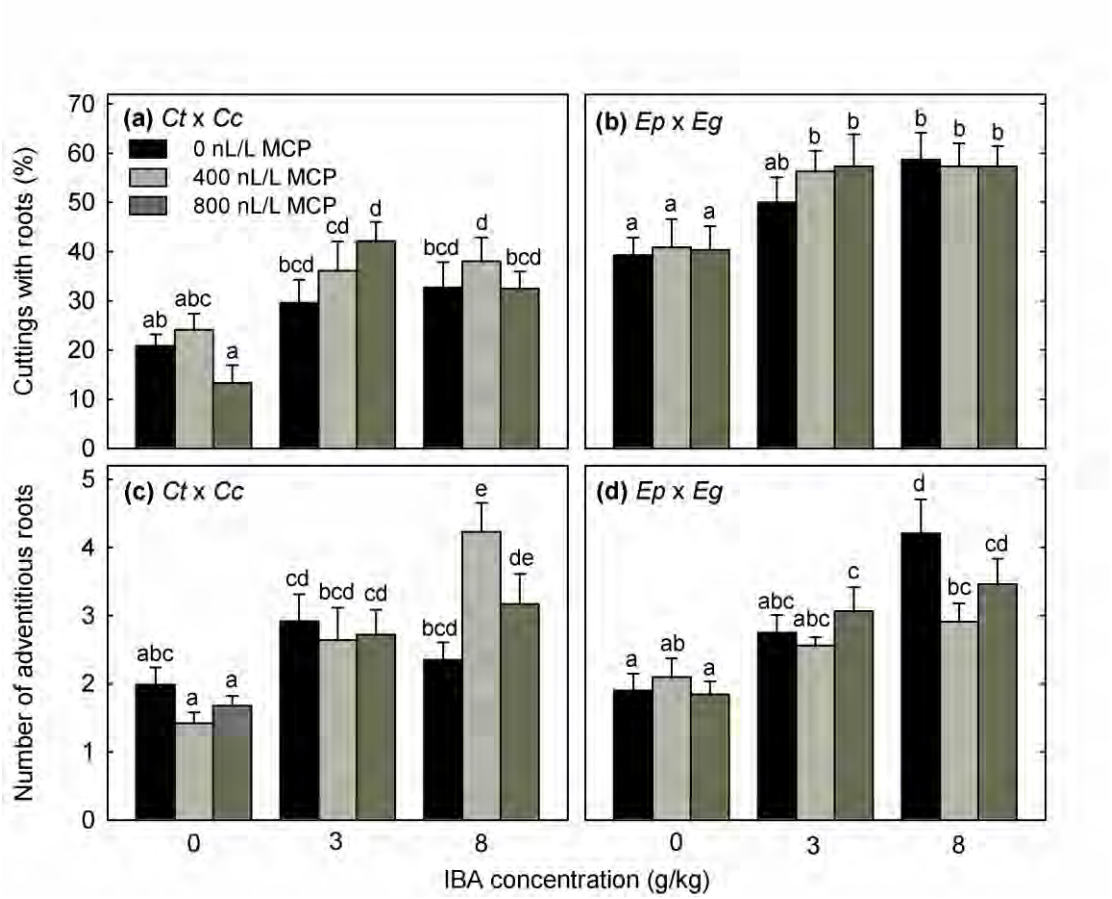

Fig. 2.16. Experiment 1: Percentage of cuttings with roots (a, b) and number of adventitious roots per rooted cutting (c, d) for *Corymbia torelliana* × *C. citriodora* (*Ct* × *Cc*) and *Eucalyptus pellita* × *E. grandis* (*Ep* × *Eg*) cuttings subjected to one of nine combinations of IBA (0, 3 or 8 g/kg) and MCP (0, 400 or 800 nL/L). Means (+ s.e.) with different letters within a hybrid are significantly different (ANOVA and Duncan's multiple range test, *P* < 0.05, n = 10)

Two of the combined IBA/MCP treatments, 8 g/kg IBA with 400 or 800 nL/L MCP, significantly increased the root weights of *C. torelliana* × *C. citriodora* rooted cuttings (Table 2.6). Root weights were also increased significantly when cuttings were treated with 3 g/kg IBA and no MCP (Table 2.6). Shoot weight and total weights of *C. torelliana* × *C. citriodora* cuttings, and root, shoot and total weights of *E. pellita* × *E. grandis* cuttings, did not differ significantly among the nine treatments (Table 2.6).

Table 2.6. Root, shoot and total dry weights of rooted cuttings of *Corymbia torelliana* × *C. citriodora* and *Eucalyptus pellita* × *E. grandis* subjected to one of nine IBA and MCP treatments

IBA (g/kg)	MCP (nL/L)	Root weight (mg)	Shoot weight (mg)	Total weight (mg)
C. torelliana × *C. citriodora*				81.2 ± 14.9 *
0	0	12.1 ± 1.7 ab	83.8 ± 11.8	95.9 ± 12.7
0	400	13.3 ± 2.8 ab	95.7 ± 12.8	109.0 ± 15.3
0	800	8.8 ± 2.3 a	70.4 ± 15.9	79.2 ± 18.1
3	0	22.1 ± 2.7 c	101.2 ± 11.2	123.3 ± 12.5
3	400	16.2 ± 3.2 abc	78.6 ± 16.4	94.8 ± 19.4
3	800	19.7 ± 3.8 bc	99.1 ± 14.8	118.8 ± 18.3
8	0	16.5 ± 2.9 abc	65.4 ± 13.2	81.9 ± 15.7
8	400	23.7 ± 2.0 c	94.2 ± 9.4	117.9 ± 10.4
8	800	24.7 ± 2.7 c	91.1 ± 11.5	115.8 ± 13.7
E. pellita × *E. grandis*				28.8 ± 5.0 *
0	0	11.9 ± 2.3	55.7 ± 8.0	67.6 ± 9.2
0	400	9.9 ± 1.7	53.4 ± 7.3	63.3 ± 8.7
0	800	8.8 ± 1.4	54.6 ± 8.2	63.4 ± 8.7
3	0	12.3 ± 1.3	56.5 ± 5.3	68.8 ± 8.5
3	400	16.2 ± 1.3	59.5 ± 4.2	75.7 ± 4.3
3	800	17.3 ± 4.5	63.8 ± 9.2	81.1 ± 13.7
8	0	18.5 ± 2.4	58.1 ± 5.1	76.6 ± 6.9
8	400	14.7 ± 1.9	52.2 ± 4.9	66.9 ± 5.8
8	800	17.8 ± 3.3	61.7 ± 8.1	79.5 ± 11.4

Means (± s.e.) with different letters are significantly different (ANOVA and Duncan's multiple range test; $P < 0.05$; n = 7-10 for *C. torelliana* × *C. citriodora*; n = 10 for *E. pellita* × *E. grandis*).

*Initial weight of cuttings

2.2.2.3.2 Experiment 2: AVG concentration

The final percentage of *C. torelliana* × *C. citriodora* cuttings displaying leaf abscission was higher for cuttings treated with 3 or 8g/kg IBA without AVG (60.7 ± 4.5% and 64.9 ± 7.1%, respectively, compared with 46.0 ± 4.2% for untreated cuttings; Fig. 2.17c). This abscission was alleviated by the application of 125 or 250 mg/L AVG, which reduced the percentage of defoliated cuttings to 46.4 ± 4.4% and 44.0 ± 6.3%, respectively, for cuttings treated with 3 g/kg IBA, and to 46.6 ± 4.7% and 40.3 ± 5.3%, respectively, for cuttings treated with 8 g/kg IBA (Fig. 2.17c). Two treatments, 8 g/kg IBA with 0 or 125 mg/L AVG, resulted in higher mortality (9.3 ± 2.8% and 10.0 ± 3.2%) than for cuttings treated with 0 g/kg IBA and 125 or 250 mg/L AVG (0.7 ± 0.7% and 2.0 ± 1.4%) (Fig. 2.17e). Leaf abscission and cutting death did not vary significantly among the nine treatments for *E. pellita* × *E. grandis* (Figs 2.17d, 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* (Figs 2.17c, d).

The percentage of *C. torelliana* × *C. citriodora* cuttings that formed roots was significantly increased, compared with untreated cuttings, when the cuttings were treated with 3 g/kg IBA with or without AVG (Fig. 2.18a). However, rooting was only increased using 8 g/kg IBA if the IBA application was followed by an application of 125 or 250 mg/L AVG (Fig. 2.18a). The percentages of cuttings that formed roots following these treatments were 21.3 ± 4.5% – 39.3 ± 3.9%, whereas only 12.0 ± 3.7% of untreated cuttings formed roots. For *E. pellita* × *E. grandis*, three of the treatments, 3 g/kg IBA with 0 or 250 mg/L AVG, and 8 g/kg IBA with 125 mg/L AVG, significantly increased the percentage of cuttings with roots compared with untreated cuttings (Fig. 2.18b). The percentages of *E. pellita* × *E. grandis* cuttings that formed roots following these treatments were 34.3 ± 4.7 – 40.7 ± 5.4%, compared with 19.4 ± 4.6% of untreated cuttings.

The number of adventitious roots per rooted *C. torelliana* × *C. citriodora* cutting was increased following application of 8 g/kg IBA with or without AVG, or following application of 3g/kg IBA with 250 mg/L AVG (Fig. 2.18c). The numbers of roots following these treatments were between 3.5 ± 0.5 and 4.3 ± 0.7, compared with 1.6 ± 0.2 for untreated cuttings (Fig. 2.18c). No treatment significantly affected the number of adventitious roots per rooted cutting, when compared with untreated cuttings, for *E. pellita* × *E. grandis* (Fig. 2.18d).

One of the combined IBA/AVG treatments, 8 g/kg IBA with 250 mg/L AVG, significantly increased the root weights of *C. torelliana* × *C. citriodora* cuttings (Table 2.7). The shoot and total weights of *C. torelliana* × *C. citriodora* cuttings, and the root, shoot and total weights of *E. pellita* × *E. grandis* cuttings, did not differ significantly among treatments (Table 2.7).

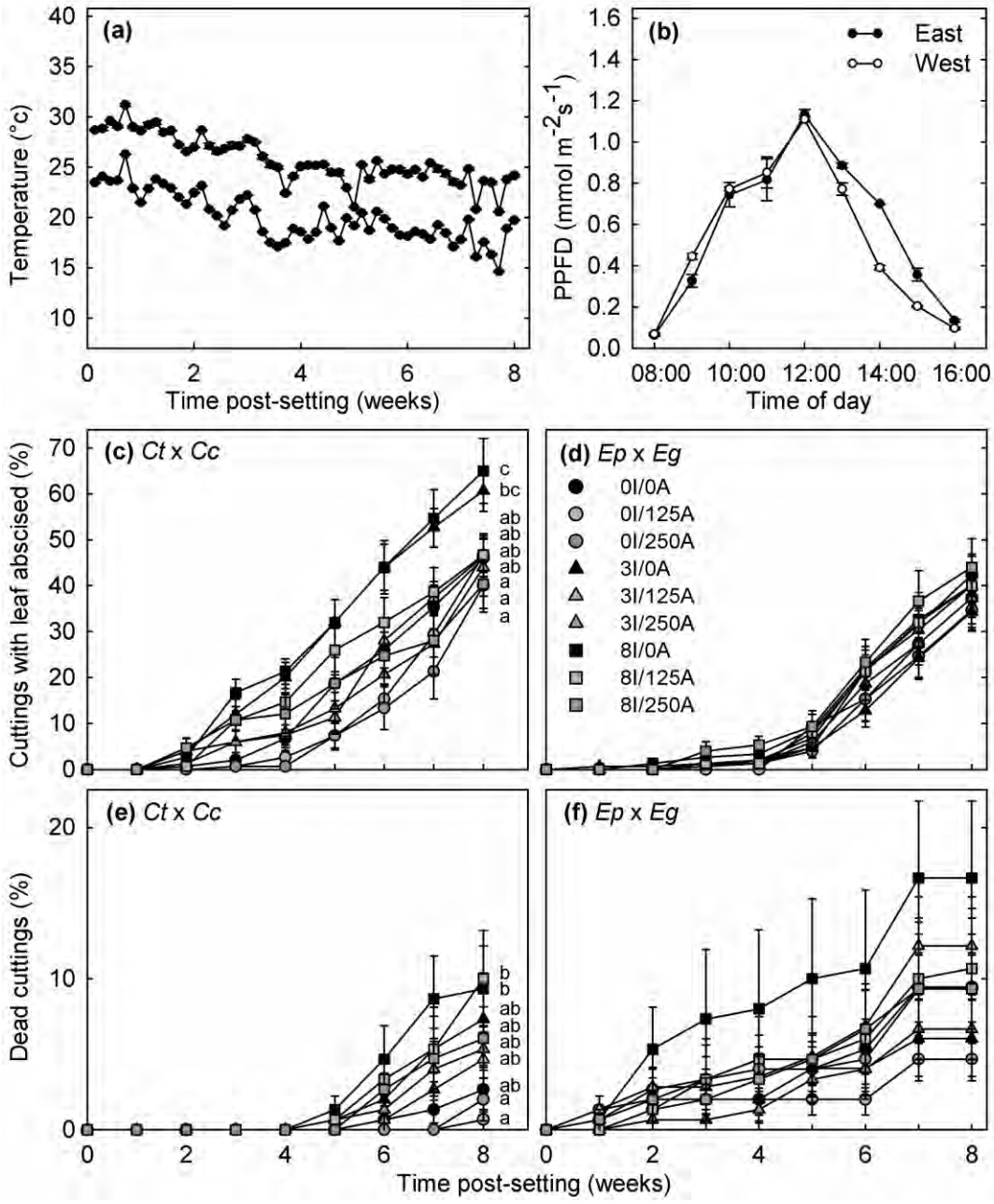

Fig. 2.17. Experiment 2: Daily maximum and minimum temperatures (a) and photosynthetic photon flux densities (PPFD) (b) in the glasshouse, and percentage of cuttings with the leaf abscised (c, d) and mortality (e, f) for *Corymbia torelliana* × *C. citriodora* (*Ct* × *Cc*) and *Eucalyptus pellita* × *E. grandis* (*Ep* × *Eg*) cuttings subjected to one of nine combinations of IBA (I) (0, 3 or 8 g/kg) and AVG (A) (0, 125 or 250 mg/L). Final means (+ s.e.) with different letters within a hybrid are significantly different (ANOVA and Duncan's multiple range test, $P < 0.05$, n = 10)

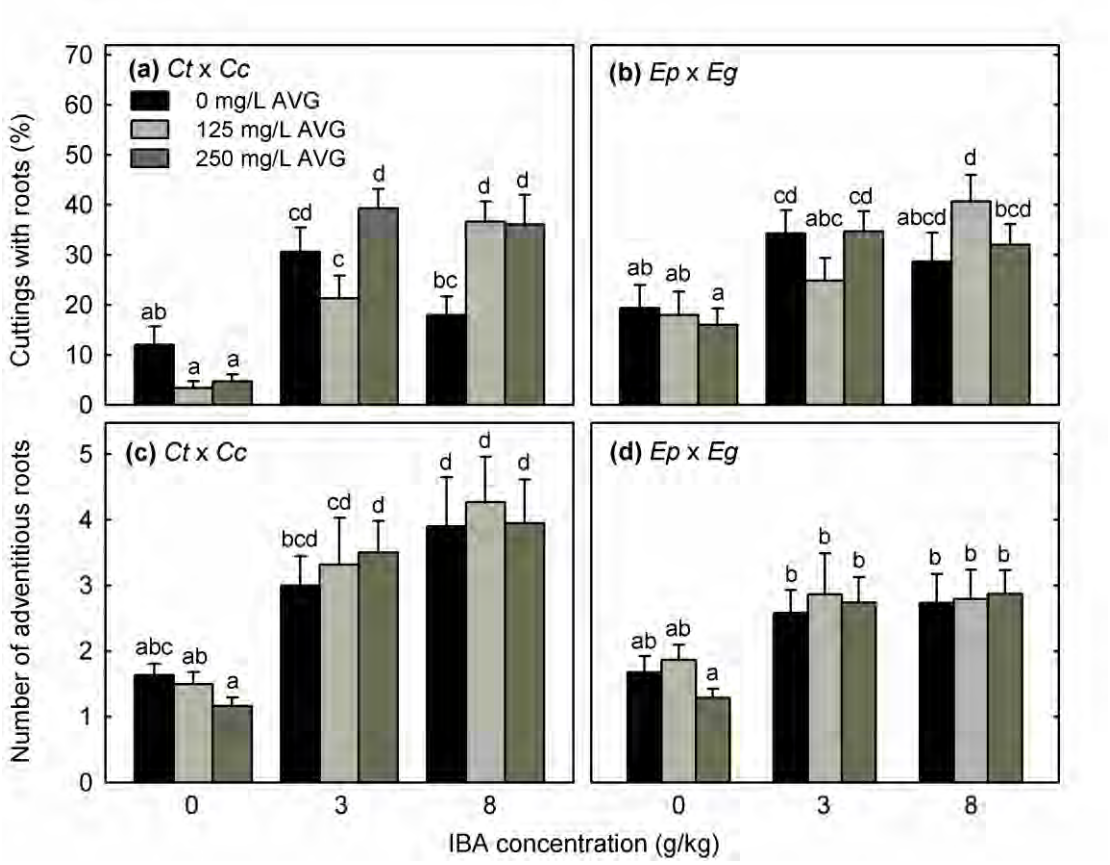

Fig. 2.18. Experiment 2: Percentage of cuttings with roots (a, b) and number of adventitious roots per rooted cutting (c, d) for *Corymbia torelliana* × *C. citriodora* (*Ct* × *Cc*) and *Eucalyptus pellita* × *E. grandis* (*Ep* × *Eg*) cuttings subjected to one of nine combinations of IBA (0, 3 or 8 g/kg) and AVG (0, 125 or 250 mg/L). Means (+ s.e.) with different letters within a hybrid are significantly different (ANOVA and Duncan's multiple range test, *P* < 0.05, n = 4-10 for *C. torelliana* × *C. citriodora* and 7-10 for *E. pellita* × *E. grandis*)

2.2.2.3.3 Timing of adventitious root formation

Adventitious roots were evident microscopically within 14 days of setting for both C. *torelliana* × *C. citriodora* and *E. pellita* × *E. grandis* (Table 2.8). 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 (Plate 2.8a, b). Adventitious root primordia arose at or near the cambium (Plate 2.8c, d). Adventitious roots emerged through the epidermis by 14 days after setting in both C. *torelliana* × *C. citriodora* and *E. pellita* × *E. grandis* (Table 2.8; Plate 2.8e, f).

Table 2.7. Root, shoot and total dry weights of rooted cuttings of *Corymbia torelliana* × *C. citriodora* and *Eucalyptus pellita* × *E. grandis* subjected to one of nine IBA and AVG treatments

IBA (g/kg)	AVG (mg/L)	Root weight (mg)	Shoot weight (mg)	Total weight (mg)
C. torelliana × *C. citriodora*				48.2 ± 6.7 *
0	0	30.5 ± 6.8 b	109.5 ± 19.3	140.0 ± 25.2
0	125	9.5 ± 0.9 a	68.8 ± 16.5	78.3 ± 17.2
0	250	32.5 ± 5.9 b	117.7 ± 15.7	150.2 ± 20.2
3	0	45.9 ± 5.6 bc	113.6 ± 15.2	159.5 ± 20.4
3	125	38.6 ± 2.2 bc	106.4 ± 11.3	145 ± 12.0
3	250	41.0 ± 6.1 bc	105.0 ± 12.3	146.0 ± 17.5
8	0	46.9 ± 5.6 bc	102.8 ± 11.1	149.7 ± 15.6
8	125	47.5 ± 3.4 bc	84.1 ± 8.4	131.6 ± 9.8
8	250	51.5 ± 2.7 c	122.3 ± 11.4	173.8 ± 12.7
E. pellita × *E. grandis*				46.4 ± 9.7 *
0	0	10.3 ± 2.3	51.6 ± 10.0	61.9 ± 11.8
0	125	15.1 ± 2.0	67.0 ± 8.5	82.1 ± 10.4
0	250	14.9 ± 3.1	60.9 ± 11.6	75.8 ± 14.6
3	0	30.5 ± 6.8	90.1 ± 18.6	120.6 ± 25.0
3	125	21.9 ± 2.4	71.3 ± 14.0	93.2 ± 14.8
3	250	22.8 ± 3.6	66.4 ± 7.8	89.2 ± 10.5
8	0	34.7 ± 7.4	65.6 ± 11.7	100.3 ± 18.9
8	125	26.4 ± 3.6	51.3 ± 7.6	77.7 ± 9.1
8	250	26.2 ± 8.4	70.9 ± 13.9	97.1 ± 20.9

Means (± s.e.) with different letters are significantly different (ANOVA and Duncan's multiple range test; $P < 0.05$; n = 4-10 for *C. torelliana* × *C. citriodora*; n = 8-10 for *E. pellita* × *E. grandis*)

*Initial weight of cuttings

Table 2.8. Timing of adventitious root initiation and emergence in *Corymbia torelliana* × *C. citriodora* and *Eucalyptus pellita* × *E. grandis* cuttings

Days ex-setting	IBA (g/kg)	*C. torelliana* × *C. citriodora*	*E. pellita* × *E. grandis*	*C. torelliana* × *C. citriodora*	*E. pellita* × *E. grandis*
		Cuttings with root initiation		Cutting with root emergence	
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

Frequencies of observations from five samples are provided

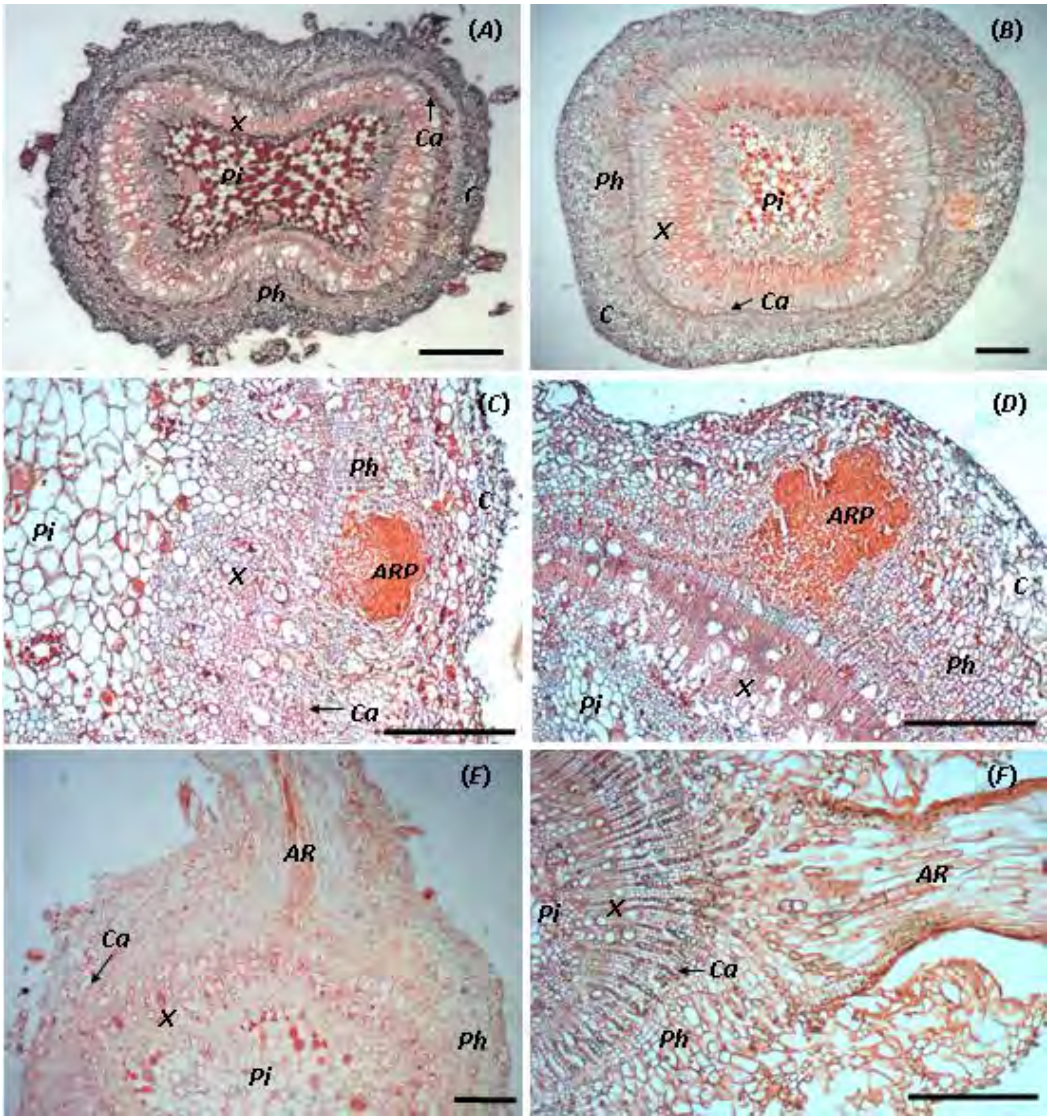

Plate 2.8. Stem anatomy at the base of eucalypt cuttings (transverse sections): (a) *Corymbia torelliana* × *C. citriodora* at 21 days after setting (0 g/kg IBA); (b) *Eucalyptus pellita* × *E. grandis* at 14 days after setting (0 g/kg IBA). Adventitious root primordia in the base of eucalypt cuttings (transverse sections): (c) Adventitious root primordium in *Corymbia torelliana* × *C. citriodora* at 14 days after setting (0 g/kg IBA); (d) Adventitious root primordium in *Eucalyptus pellita* × *E. grandis* at 14 days after setting (0 g/kg IBA). Emergence of adventitious roots from the base of eucalypt cuttings (transverse sections): (e) Adventitious root emerging from *C. torelliana* × *C. citriodora* at 14 days after setting (8 g/kg IBA); (f) Adventitious root emerging from *E. pellita* × *E. grandis* at 21 days after setting (0 g/kg IBA). Scale bars = 150 µm. *AR* = adventitious root, *ARP* = adventitious root primordium, *Ca* = cambium, *C* = cortex, *Pi* = pith, *Ph* = phloem, *X* = xylem

2.2.2.4 Discussion: The optimal level of rooting hormone for propagation of the hybrids, C. torelliana × C. citriodora and E. pellita × E. grandis

This project has developed improved propagation techniques for these eucalypt hybrids. 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 increased defoliation and death of *C. torelliana* × *C. citriodora* cuttings in one experiment, but AVG alleviated these effects and also increased the percentage of cuttings that formed roots. The most effective treatments for increasing rooting

were often the combined IBA and MCP or AVG treatments, particularly for *C. torelliana* × *C. citriodora*. There appears ample opportunity for MCP or AVG application after IBA application but prior to defoliation because root initiation and emergence were already evident within 14 d of setting, well before the main phases of cutting defoliation and death.

IBA application in the absence of MCP or AVG had promotive, though inconsistent, effects on the percentage of cuttings with roots and the number of adventitious roots per rooted cutting. Both 3 and 8 g/kg IBA increased the percentage of cuttings with roots in one experiment but not the other, and 8 g/kg IBA increased the number of roots per rooted cutting in one experiment but not the other, for both *C. torelliana* × *C. citriodora* and *E. pellita* × *E. grandis*. IBA has increased the percentage of *C. torelliana*, *E. grandis*, *E. grandis* × *E. urophylla*, *E. nitens* and *E. pellita* × *E. tereticornis* cuttings that forms roots in previous studies. However, high IBA doses are less effective than intermediate doses in *E. grandis* × *E. urophylla*, *E. pellita* × *E. tereticornis* and *C. torelliana* × *C. citriodora*. High IBA doses can also reduce rooting percentages in *E. grandis, E. grandis* × *E. urophylla* and *C. citriodora*.

The highest dose of IBA (8 g/kg) significantly increased defoliation and death of *C. torelliana* × *C. citriodora* cuttings in one Experiment (2). Defoliation commenced in the second week after setting. Defoliation has been observed previously at this dose for *C. citriodora*, *C. torelliana* and *C. torelliana* × *C. citriodora* cuttings, and defoliation becomes evident in tissue cultures of *C. citriodora* and *C. maculata* when shoots are induced to form roots by NAA or IBA, respectively. The present findings, that IBA-induced leaf abscission and death of *C. torelliana* × *C. citriodora* cuttings were alleviated by AVG application, and that AVG elevated the percentage of cuttings that formed roots, indicate that auxin-stimulated defoliation of *Corymbia* cuttings is caused by ethylene production and that high ethylene production is detrimental to rooted cuttings 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 the result of ethylene production. Indeed, MCP had no effect on defoliation during the Experiment (1) in which IBA did not induce defoliation.

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. 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 d after setting for both *C. torelliana* × *C. citriodora* and *E.*

pellita × *E. grandis* cuttings. 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. The precise site of initiation was not evident from the sections obtained in the current project, but the approximate location at or near the vascular cambium is consistent with the sites in many other woody species.

The combined IBA and MCP or AVG treatments improved the percentage of cuttings that formed roots, the number of adventitious roots per rooted cutting and also, occasionally, the subsequent root weight. For example, the combination of high IBA (8 g/kg) with high MCP (800 nL/L) or AVG (250 mg/L) increased root weight of *C. torelliana* × *C. citriodora* cuttings by 104% and 69% in Experiments 1 and 2, respectively. Nil-hormone treated cuttings of hardwood species often produce very few roots, and higher root number, weight or volume can aid root system symmetry, plant survival, tree stability, tree height or stem diameter.

2.2.2.5 Conclusion: The optimal level of rooting hormone for propagation of the hybrids, C. torelliana × C. citriodora and E. pellita × E. grandis

We recommend that cuttings of *Corymbia torelliana* × *Corymbia citriodora* subsp. *variegata* and *Eucalyptus pellita* × *Eucalyptus grandis* be treated with an intermediate dose of rooting hormone (3 g IBA / kg powder) to maximise the production of rooted cuttings.

This project has developed improved propagation techniques for the eucalypt hybrids, *C. torelliana* × *C. citriodora* and *E. pellita* × *E. grandis*. Treatments that combined the rooting hormone with an ethylene inhibitor also markedly increased rooted cuttings production and improved root system quality. For instance, combining IBA (8 g/kg) with MCP (400 nL/L) or AVG (125 mg/L) 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. Deployment of these *Corymbia* and *Eucalyptus* hybrids is, therefore, possible through a vegetative family forestry or clonal 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.

Chapter 3. Potential gene flow risks from *Corymbia* plantations

3.1 Pollen-mediated gene flow risks from *Corymbia* hybrid plantations

3.1.1 Introduction

Corymbia species and their hybrids are of increasing importance to plantation forestry due to their wide adaptability to marginal environments and high quality, durable timber (Carvalho et al., 2010; Lee, 2007; Lee et al., 2010, Nichols et al., 2010). Over 20,000 hectares of *Corymbia* plantations have been established across Australia since the late 1990's (Barbour et al., 2008), primarily using *C. citriodora* subsp. *variegata* (CCV). The expansion of *Corymbia* plantations on a greater scale has been impeded by susceptibility to the pathogen *Quambalaria pitereka* (Brawner et al., 2011; Dickinson et al., 2004; Pegg et al., 2009) and low productivity rates (Lee et al., 2010; 2011). *Corymbia* hybrids offer advantages over their parental species, such as superior growth, disease, insect, and frost tolerance, exhibited across a wide range of environments (Lee, 2007; Lee et al., 2009; Nahrung et al., 2010; 2011). These hybrids are derived by controlled pollination using *C. torelliana* (section *Torellianae*) as the maternal taxon and species from the spotted gum group (section *Maculatae*); *C. citriodora* subsp. *citriodora* (CCC), CCV or *C. henryi*, as the paternal taxon (Dickinson et al., 2010; Lee, 2007). *Corymbia* hybrid breeding is currently focussed on a range of F1 hybrid families derived from these three interspecific hybrid combinations (Lee *et* al., 2009) or from new interspecific hybrid combinations involving different parental species (Dickinson et al., 2012).

Infrageneric *Corymbia* clades are closely related (Hill and Johnson, 1995; Parra-O et al., 2009) and have an unusually high propensity to form interspecific hybrids across taxonomic groups (Dickinson et al., 2012; Griffin et al., 1988). This poses a risk of pollen-mediated gene flow from plantation populations into sympatric native *Corymbia* populations. Gene flow into native *Corymbia* populations is considered a high risk for CCC and CCV plantations and a moderate risk from *C. torelliana* × *C. citriodora* F1 hybrid plantations in eastern Australia (Barbour et al., 2008). There are further risks of gene flow from *C. torelliana* due to the unusual long-distance seed dispersal mechanism, seed dispersal by bees (Wallace and Trueman 1995*,* Wallace et al., 2008 Wallace and Lee 2010). Reproductive success in other taxa can be greater when F1 hybrids are backcrossed with either parental species, as many isolating barriers have been circumvented during F1 hybridisation (Arnold et al., 1999; Potts et al., 2000)., Eucalypt plantations have expanded rapidly in Australia increasing the threat of genetic pollution via pollen-mediated gene flow into sympatric native populations (Barbour et al., 2006, 2008, 2010; Potts et al., 2003). A better understanding of the breeding system and

biology of *Corymbia* plantations is required to minimise future gene flow risks (Barbour et al., 2008).

Interspecific hybridisation results in gene flow between individuals and contributes to plant speciation (Arnold et al., 1999; Arnold and Martin, 2010; Potts et al., 2003). Environmental and endogenous isolating barriers inhibit hybridisation and maintain species integrity (Potts and Wiltshire, 1997). Endogenous barriers to reproduction increase with greater taxonomic distance between parents (Ellis et al., 1991; Griffin et al., 1988). Endogenous reproductive isolating barriers may be structural; such as disparity in flower morphology of the parent species (Gore et al., 1990) or physiological; whereby genetic combining irregularities between the parental species interrupt or impede reproduction and development (Dickinson et al., 2012; Ellis et al., 1991, Pound et al., 2002, 2003; Suitor et al., 2008; Wallwork and Sedgley, 2005). Reproductive isolation can continue after seeds are produced resulting in mortality and abnormal growth at germination, during seedling development and as trees (Barbour et al., 2006; Volker et al., 2008). Interspecific reproductive isolation between *C. torelliana* (CT) and other species is primarily controlled by prezygotic isolating barriers (Dickinson et al., 2012); however, reduced hybrid fitness and survival after germination amongst CT F1 hybrid families is also common (Lee et al., 2009). Little is known about the mechanisms of reproductive isolation and the relative success of reciprocal and advanced generation CT hybrids.

This study examines the reproductive success of reciprocal *C. c. citriodora* and *C. torelliana* hybrids*,* and advanced generation hybrids where both parental species were back-crossed with CT×CCC or CT×CCV F1 hybrid taxa. Pollen-pistil interactions, embryo development and seed germination stages were examined, to determine the effects of reproductive isolating barriers at prezygotic, postzygotic and seedling development stages. This study provides information on the reproductive biology of CCC*,* CT and their F1 hybrids, identifies opportunities for tree breeding and raises implications for pollen-mediated gene flow risk into native *Corymbia* populations.

3.1.2 Experiment 1: Locations of Interspecific Reproductive Isolating Barriers

3.1.2.2 Methods and Materials

Experiment 1 was conducted from 2007–2008, to identify the location of barriers to interspecific reproductive success via pollen, pollen tube, embryo and seed measurements CT was used as the maternal parent and was crossed with the selected pollen parents: CT*,* CCC*, C. tessellaris* (CTess) and *C. intermedia*, representing four *Corymbia* sections. The two

maternal parent trees used in the experiment were large (>8 m tall) and were selected from amenity plantings of unknown genetic origin, near Mareeba, 17.00°S, 145.43°E, Queensland, Australia. Pollen from each paternal parent was collected from two to six individuals per taxa. Flowers were collected prior to opening, placed in vases in the laboratory and anthers harvested after operculum shedding. The pollen was then extracted, dried for 72 hours in a silica-gel desiccator and stored in gel capsules at 4°C until required. A pollen polymix was made for each parent species, with pollen viability confirmed two weeks prior to pollination, using the methods described by Moncur (1995).

Controlled pollinations were carried out between August and October, using the conventional pollination method (Van Wyk, 1977; Dickinson et al., 2010). All flowers were accessed using an 8 m elevated platform. Each cross treatment was conducted on three flower bunches on each of the two maternal parents. Samples were collected one and five weeks after pollination by randomly harvesting 3–4 capsules per bunch. These were pooled to give 10 samples per cross treatment for each maternal parent, thus giving twenty replicates per harvest. All remaining capsules for each maternal parent were harvested at maturity and assessed individually. Flower bunches were selected prior to pollination, if most buds were yellow and within 0–3 days of natural operculum lift. Open flowers and immature, overripe and excessive buds were then removed, retaining approximately 50–100 buds per bunch. The retained flowers were emasculated using pollination pliers and then covered with an exclusion bag. They were pollinated approximately seven days later when the stigmas had visible exudate. Exclusion bags were re-applied and retained for a further seven days, then removed. Pollinated flowers were covered with a polyester exclusion bag for 14 days, to prevent pollen contamination from other sources.

Samples were collected one week after pollination for measurement of pollen adhesion and germination, pollen tube growth and embryo fertilisation. Ten flowers per cross treatment were collected for each maternal parent, fixed in Carnoy's solution (60% ethyl alcohol [95%], 30% chloroform, 10% glacial acetic acid), and stored at 4°C, until assessment. Samples were rinsed in distilled water three times then softened by autoclaving at 121°C for 20 minutes in a solution of 0.8 N NaOH. Samples were then rinsed in distilled water and stained in a solution of decolourised aniline blue (DAB), for a minimum of 48 hours. Pistils were dissected from the developing capsule and cut longitudinally using a scalpel blade, exposing the transmitting tissue and pollen tubes for pollen tube measurement. Pistils were then squashed onto slides with the cut surface of both halves facing upright and viewed under fluorescence using a Zeiss Axioskop (2 MOT) microscope. A sub-section comprising 33% of the stigma surface was examined for each sample, the number of germinated and non-germinated pollen grains counted (Plate 3.1A) and germination percentage calculated. The

transmitting tissue in the middle section of the style was then examined (Plate 3.1B) and the number of pollen tubes counted. One of the three locules in each flower was randomly selected and the ovules dissected and counted using a stereo microscope. Ovules were then mounted on a slide and viewed using fluorescent microscopy. Fertilised ovules were identified where pollen tubes were observed to have penetrated the ovule micropyle (Plate 3.1C).

Ten developing capsules per cross treatment were harvested at five weeks after pollination, fixed in FPA50 (90% ethyl alcohol [50%], 5% formalin, 5% propionic acid) and stored at 4°C until required. Capsules were dissected and the number and size of developing embryos were assessed. Developing embryos were distinguished from other embryos by size (> 700 µm) and appearance, with developing embryos well hydrated and light brown/yellow in colour (Plate 3.1D). Capsules were harvested at maturity 12 weeks after pollination, dried individually, seed extracted and the seed number per capsule measured. At maturity, capsules were harvested and air-dried for a minimum of seven days. Seed was then extracted and the seed number per capsule calculated. The results of capsule retention at maturity and seed number per capsule were used to calculate seed number produced per capsule pollinated. Seed viability was assessed, with available seed (maximum of 100 seeds per bunch) sown onto germination trays, placed into a germination cabinet and incubated at 25°C for 10 days. The germinated seed were then counted and viable seed per capsule pollinated was calculated.

Prior to analysis, all data were screened for assumptions of normality and homogeneity of variance. Where necessary, percentage data were arcsine-transformed to meet the assumptions of parametric tests and numerical data were log-transformed to correct for unequal variances. Statistical analysis was conducted using Genstat 9.2 statistical software (Genstat, 2007). For all experiments, data were analysed via analysis of variance (ANOVA). Where F values were significantly different ($P < 0.05$), pair-wise comparisons between means were conducted using Tukey's multiple comparison test.

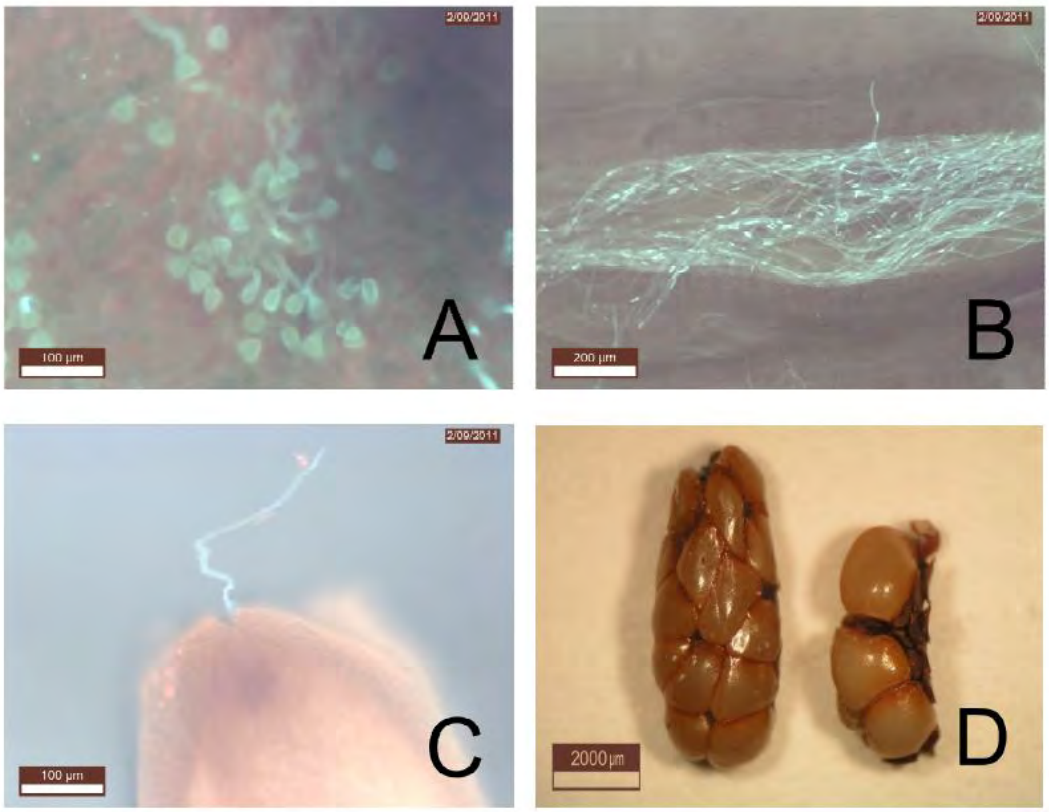

Plate 3.1. Fluorescent microscope images; (A) germinating pollen on the stigma surface, CT×CCC, scale bar = 100 µm, (B) pollen tubes in mid stylar region, CT×CCC, scale bar = 200 µm and (C) pollen tube penetration of the ovule micropyle, CT×CT scale bar = 100 µm. Light microscope image; (D) developing embryos within the locule at age five weeks, CT× and CT×CTess (right), scale bar = 2000 µm.

3.1.2.3 Results

Prezygotic reproductive isolating barriers were observed within the CT maternal parent at all four prezygotic fertilisation stages, with significant differences ($P < 0.05$) between crosses for the parameters of pollen adhesion to stigma, pollen germination, pollen tube growth through the style and pollen tube penetration of the ovule micropyle. Differences in reproductive success between crosses were immediately apparent at the first stage of fertilisation, with the number of pollen grains that adhered to the stigma for the interspecific *C. intermedia* cross significantly lower ($P < 0.05$) than the intraspecific CT cross A further barrier to reproductive success was observed at the second stage of fertilisation, with the germination percentage of adhered pollen grains on the stigma surface significantly lower ($P < 0.05$) for the *C. intermedia* cross than the *C. torelliana* cross. There were no differences in pollen to stigma adhesion or pollen germination between the CT, CCC and *C. tess* crosses (Table 3.1).

The number of pollen tubes observed in the middle style region for the interspecific *C. intermedia* and *C. tess* crosses was significantly lower ($P < 0.001$) than the intraspecific CT cross. Pollen tube numbers were not significantly different between the CCC and CT

crosses. At the fourth stage of fertilisation, the number of ovules penetrated by pollen tubes was lowest ($P < 0.001$) for the *C. intermedia* and *C. tess* crosses, intermediate for the CCC cross and highest for the CT cross (Table 3.1).

Five weeks after pollination, the intraspecific CT cross had significantly more developing embryos per capsule ($P < 0.01$) than the interspecific CCC, CT and *C. intermedia* crosses (Table 3.1). The CCC cross also had a significantly higher number of developing embryos than the CT and *C. intermedia* crosses. Embryo size at 5 weeks after pollination was greatest in crosses where embryo numbers were low (Table 3.1). Embryos within the *C. intermedia* and CT crosses were significantly larger ($P < 0.001$) than embryos within the CT and CCC crosses (Table 3.1). At capsule maturity, the CT cross had a significantly higher ($P < 0.001$) seed number per capsule than all crosses The CCC cross also had significantly higher seed yields than the *C. tess* and *C. intermedia* crosses. Seed yields for both the *C. intermedia* and CT crosses were very low and not significantly different from each other (Table 3.1).

Table 3.1. Prezygotic reproductive success for pollen adhesion, germination, pollen tube growth and ovule penetration, (seven days after pollination) and postzygotic reproductive success for embryo development (five weeks after pollination) and seed production (twelve weeks after pollination), for the four crosses made with the *C. torelliana* maternal parent in experiment 1. Treatment means with different letters are significantly different ($P < 0.05$).

Pollen parent	Subgenus, section	Prezygotic				Postzygotic		
		Pollen grains on stigma	Pollen germination	Pollen tubes in style	Ovules penetrated	Embryos / capsule	Embryo size (mm)	Seeds / capsule
CT	*Blakella, Torellianae*	326 a	51.3% a	62.9 a	37.8 a	39.9 a	1.44 b	35.3 a
CCC	*Blakella, Maculatae*	223 ab	55.7% a	47.9 ab	24.2 b	23.2 b	1.48 b	23.1 b
CTess	*Blakella, Abbreviatae*	275 ab	49.4% ab	40.4 b	10.8 c	2.9 c	1.91 a	4.3 c
C. intermedia	*Corymbia, Septentrionales*	160 b	37.9% b	9.9 c	2.3 c	0.2 c	1.88 a	1.8 c
P value		0.033	0.030	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001

3.1.2.4 Discussion: Locations of Interspecific Reproductive Isolating Barriers

Interspecific CT hybridisation was controlled by prezygotic reproductive isolating barriers. Reproductive isolation occurred immediately for the *C. intermedia* cross, with few pollen grains adhering to the stigma. Pollen adhesion is the first cellular event that occurs when pollen is deposited on the stigma (Heizmann et al., 2000) and is reliant on a successful interaction between the pollen grain and adhesion molecules on the stigma (Heslop-Harrison, 2000; Lord, 2000). This process acts as an initial defensive mechanism preventing entry of pathogens, but can also act as a physiological isolating mechanism, which can inhibit adhesion of undesirable self pollen (Heslop-Harrison, 2000) and interspecific pollen (Rougier et al., 1988).

Low pollen germination for the *C. intermedia* cross suggests activity of another reproductive isolating barrier. Pollen germination on the stigma surface occurs after successful rehydration by watery stigma secretions (Lord, 2000) which are regulated by both physiochemical and genetic controlling factors (Heslop-Harrison, 1987). Genotypes of different compatibility hydrate at different rates, possibly determined by pollen surface molecular interactions, which results in variable pollen germination rates (Zuberi and Dickinson (1985). Optimum conditions for pollen hydration and germination vary between species within the eucalypts, particularly in the levels of lipids, proteins, carbohydrates and boric acid within the stigmatic exudate (Potts and Marsden-Smedley, 1989). Differences in pollen germination are expected for different interspecific crosses, with high variation identified between pollen parents in a *Eucalyptus* hybridisation experiment involving 21 pollen parent species (Ellis et al., 1991). In our study, the lower germination of the *C. intermedia* pollen on the CT style may be due to a physiological isolating mechanism inhibiting pollen germination on the style.

Pollen tube growth was inhibited into and throughout the style for the *C. intermedia* and CT crosses, providing further evidence of barriers to interspecific reproduction. Disruption of pollen tube growth often occurs soon after pollen germination, either during penetration of the stigma cuticle or during early growth within the transmitting tract of the style (Ellis et al., 1991; Wallwork and Sedgley, 2005). Isolation can also occur in the lower style, with pollen tube growth disrupted within the first millimetre of styles cut using the one-stop pollination method (Suitor et al., 2009). Disrupted pollen tubes are often characterised by non-directional growth and/or abnormal appearance (thickened walls, bulbous growth, forking). In eucalypts, the greater the taxonomic distance between the two parent species, the greater the chances of divergent evolution and incongruity, resulting in a greater number of malfunctions in pollen tube growth (Ellis *et al.*, 1991). Within the four *Corymbia* species investigated, pollen tube numbers in the style were lowest in the *C. intermedia* cross, intermediate in the CT cross and similar between the CCC and CT crosses.

Large differences in reproductive success were observed between crosses at the fourth stage of prezygotic fertilisation: pollen tube penetration of the ovule micropyle. Differences in reproductive output became apparent for the first time between the CT and CCC crosses and were reduced further for the CT and *C. intermedia* crosses. In eucalypts, failure of the pollen tube to penetrate the ovule micropyle is a common reproductive barrier, inhibiting self-fertilisation in *E. globulus* (Pound et al., 2002) and *E. woodwardii* (Sedgley and Smith, 1989), self and interspecific fertilisation in *E. spathulata* (Ellis and Sedgley, 1992; Sedgley and Granger, 1996) and interspecific and intergeneric fertilisation in a wide range of *Eucalyptus*

and *Corymbia* crosses (Ellis et al., 1991). Similarly in our study, inhibition of pollen tube penetration of the ovule micropyle is an important prezygotic reproductive barrier to interspecific *Corymbia* hybridisation.

We found strong evidence that reproductive isolating barriers were mostly prezygotic. Ovule and seed counts remained constant from fertilisation to maturity for the CT, CCC and *C. intermedia* crosses, with only a small reduction for the CT cross. Maintenance of species integrity, through reproductive isolation, is much more efficient at prezygotic, rather than postzygotic stages, as this conserves ovules for fertilisation by more desirable pollen and minimises wastage of plant resources via embryo abortion (Waser and Price, 1991). Reproductive isolation via the effects of incongruity and incompatibility is also likely to become greater and more immediate with increasing taxonomic distance between parents (de Nettancourt, 1984). In eucalypts, the reproductive success of interspecific crosses is more likely to be inhibited by early-acting prezygotic reproductive barriers (Ellis et al., 1991), whereas in more closely related crosses, including intraspecific out-cross and self-pollen, reproductive success is primarily controlled by late-prezygotic or postzygotic barriers (Pound *et al*., 2002).

3.1.2.5 Conclusions: Locations of Interspecific Reproductive Isolating Barriers

Interspecific *Corymbia* hybrids were successfully created between *C. torelliana* and species from both *Corymbia* subgenera, confirming the close relationships of infrageneric clades within this genus. Prezygotic isolating barriers were identified at the four stages: pollen adhesion to the stigma, pollen germination, pollen tube growth in the style and pollen tube penetration of the ovule micropyle. Postzygotic isolating barriers were not identified for any of the crosses examined. Increasing activity of reproductive isolating barriers was identified with increasing taxonomic distance between parents, suggesting interspecific incongruity is broadly applicable to *Corymbia*. The poor success of some closely related crosses suggests that factors in addition to incongruity can also influence interspecific *Corymbia* hybridisation.

3.1.3 Experiment 2: Gene Flow from Reciprocal and Advanced Generation Hybrids

3.1.3.1 Methods and Materials

Three CCC and three CT maternal parent trees were crossed with five paternal taxa cross treatments; CT, CCC, CT× CCC hybrid, CT× CCV (hybrid 1) and CT×CCV (hybrid 2) during 2007–2008. All maternal parent trees were large (>8 m tall) and were selected from amenity plantings of unknown genetic origin, near Mareeba, 17.00°S, 145.43°E, Queensland, Australia. Pollen was collected from three individuals per taxon for the CT and CCC cross treatments and one individual per taxon for the three hybrid backcross treatments. Flowers were collected prior to opening, placed in vases in the laboratory and anthers harvested after operculum shedding. The pollen was then extracted, dried for 72 hours in a silica-gel desiccator and stored in gel capsules at 4°C until required. A pollen polymix was made for the CT and CCC cross treatments. Pollen viability was confirmed two weeks prior to pollination, using the methods described by Moncur (1995).

Controlled pollination treatments were conducted on both maternal parent species between August and September, using the conventional pollination method (Moncur, 1995). All flowers were accessed using an 8 m elevated platform. Each cross treatment was conducted on three flower bunches on each maternal parent tree. Samples were collected one and five weeks after pollination by randomly harvesting 3–4 capsules per bunch. These were pooled to give 10 samples per cross treatment for each maternal taxon, thus giving thirty replicates per parameter. All remaining capsules for each maternal taxon were harvested at maturity and assessed individually as described below. Flower bunches were selected prior to pollination, if most buds were yellow and within 0–3 days of natural operculum lift. Open flowers and immature, overripe and excessive buds were then removed, retaining approximately 50–100 buds per bunch. The retained flowers were emasculated using pollination pliers and covered with an exclusion bag. Flowers were pollinated approximately seven days later when the stigmas had visible exudate. Exclusion bags were re-applied and retained for a further seven days, then removed.

Samples were collected one week after pollination for measurement of pollen tube growth and embryo fertilisation. Ten flowers per cross treatment were collected for each maternal taxon, fixed in Carnoy's solution (60% ethyl alcohol [95%], 30% chloroform, 10% glacial acetic acid), and stored at 4°C, until assessment. Samples were rinsed in distilled water three times then softened by autoclaving at 121°C for 20 minutes in a solution of 0.8 N NaOH. Samples were then rinsed in distilled water and stained in a solution of decolourised aniline blue (DAB), for a minimum of 48 hours. Pistils were dissected from the developing

capsule and cut longitudinally using a scalpel blade, exposing the transmitting tissue and pollen tubes for pollen tube measurement. Pistils were then squashed onto slides with the cut surface of both halves facing upright and viewed under fluorescence using a Zeiss Axioskop (2 MOT) microscope (Plate 3.2a and 3.2b). The transmitting tissue midway along the length of the style was examined and the number of pollen tubes counted. One of the three locules in each flower was randomly selected and the ovules dissected and counted using a stereo microscope. Ovules were then mounted on a slide and viewed using fluorescent microscopy. Fertilised ovules were identified where pollen tubes were observed to have penetrated the ovule micropyle (Plate 3.2c).

Ten developing capsules per cross treatment were harvested at five weeks after pollination, fixed in FPA50 (90% ethyl alcohol [50%], 5% formalin, 5% propionic acid) and stored at 4°C until required. Capsules were dissected and the number and size of developing embryos were assessed. Developing embryos were distinguished from other embryos by size (> 700 µm) and appearance, with developing embryos well hydrated and yellow/white in colour (Plates 3.2d and 3.2e). Mature seeds and capsules were harvested and counted; 12 weeks after pollination for CT and 21 weeks after pollination for CCC when the seeds were mature. Percentage capsule retention at maturity was calculated for each bunch and meaned per cross treatment (excluding capsules sampled at one and five weeks). Capsules were dried individually, seed extracted and the seed number per capsule measured. Seed viability was assessed for each cross treatment on each maternal tree, with 30 seeds $\times$ 3 replicates sown onto germination trays. The trays were placed into a germination cabinet and incubated at 25°C for 10 days. Germinated seed was counted and germination percentage calculated.

All data was screened for assumptions of normality and homogeneity of variance prior to analysis. Where necessary, percentage data was arcsine-transformed to meet the assumptions of parametric tests and numerical data was log-transformed to correct for unequal variances. Statistical analysis was conducted using Genstat 11.1 statistical software (Copyright 2008, VSN International Ltd). Measurement parameters for each maternal taxon were analysed separately via a general linear model using restricted maximum likelihood (REML) to estimate variance and covariance parameters. Significant differences ($P < 0.05$) between values were determined using the Wald statistical test, with pair-wise comparisons between means then conducted using the Least Significant Difference Test.

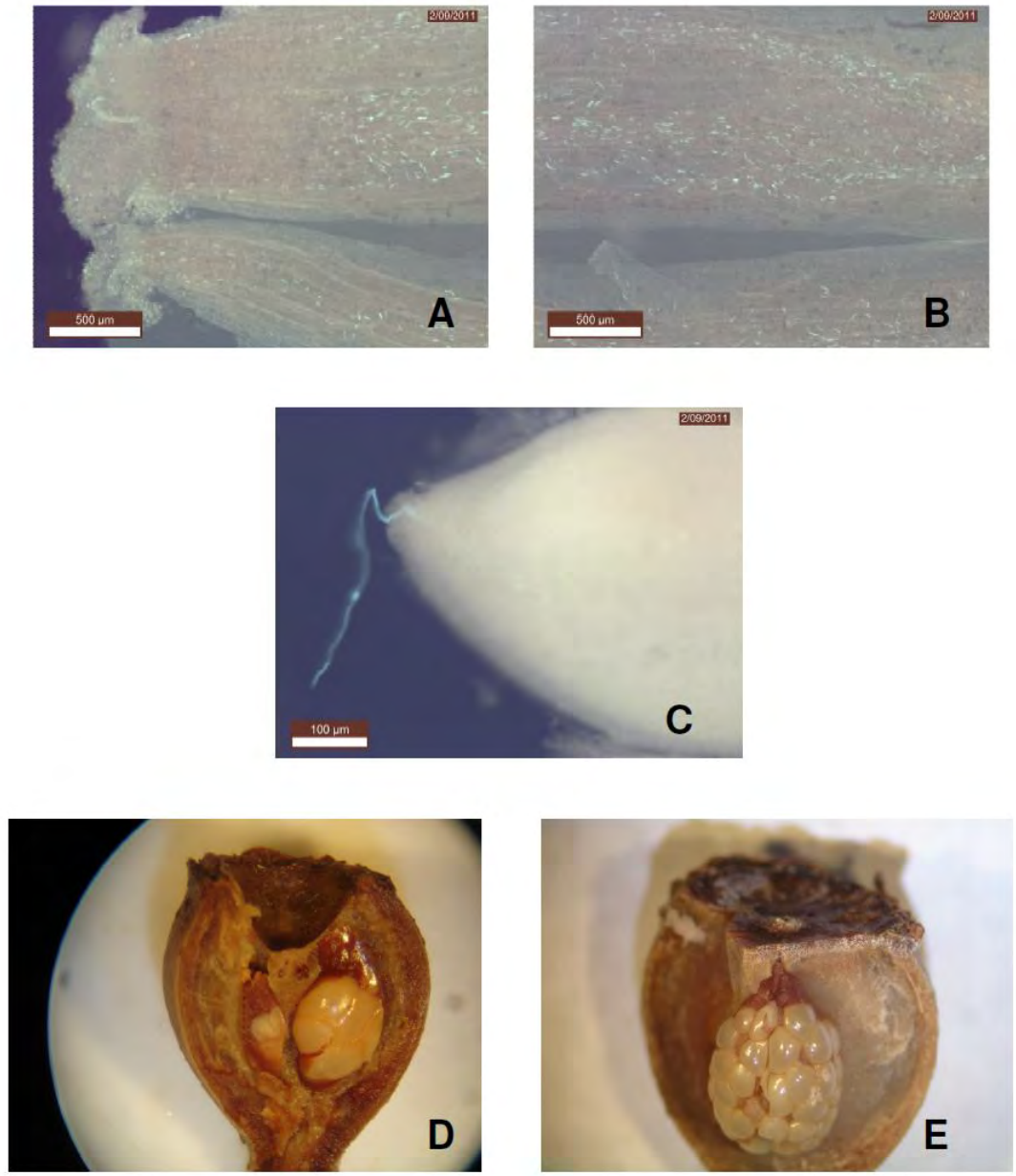

Plate 3.2. Fluorescent microscope images; (A) Germinating pollen on stigma surface and pollen tubes in upper style, *C. c. citriodora* x *C. torelliana*, scale bar = 500 µm, (B) pollen tubes in mid style, CCC×CT, scale bar = 500 µm and (C) pollen tube penetration of the ovule micropyle, CCC× (×CCC), scale bar = 100 µm. Light microscope images of ; developing embryos within the locule at age five weeks; (D) CT×CT, scale bar = 2000 µm and (E) CCC×CCC, scale bar = 2000 µm.

3.1.3.2 Results

CCC maternal taxon

High numbers of pollen tubes (45–71 per flower) were identified within the mid stylar region of the CCC maternal taxon, for all crosses examined. The most successful paternal cross treatment was the intraspecific CCC cross, which recorded a significantly higher ($P < 0.05$) number of pollen tubes than all crosses, except the CT×CCC hybrid cross (Figure 3.1A). At the next stage of fertilisation, pollen tube penetration of the ovule micropyle, high numbers of

penetrated ovules (10.2–17.7 per flower) were observed for all crosses. The highest number of penetrated ovules was observed for the intraspecific CCC cross, which was significantly higher ($P < 0.05$) than all treatments, except the CT×CCV hybrid 2 cross (Figure 3.2A).

Five weeks after fertilisation, all crosses had developing embryos (Figure 3.3A). The highest number of developing embryos was measured for the intraspecific CCC cross, which was significantly greater ($P < 0.01$) than all other crosses. There was no difference in developing embryo numbers between the other cross treatments. The size of developing embryos, measured five weeks after pollination, was not significantly different between cross treatments (Figure 3.4A).

All crosses produced seed at maturity, with a similar seed number per capsule for the CCC, CT×CCC hybrid and CT×CCV hybrid 2 crosses (Figure 3.5A). The CCC cross had a significantly greater ($P < 0.001$) seed yield than the interspecific CT cross. Poorest seed number per capsule was measured for the CT×CCV hybrid 1 cross, which was significantly lower than all other crosses. Capsule retention percentage at maturity was relatively uniform between cross treatments (23.4–34.0 %) and was not significantly different (Table 3.2). Seed viability percentage (Table 3.2) was lowest ($P < 0.01$) for the CT×CCC hybrid cross (54.2%), whereas all remaining crosses were similar (72.0–83.5 %).

C. torelliana maternal taxon

Pre-zygotic reproductive success for the CT maternal taxon was initially high across all cross treatments, with similar numbers of pollen tubes (35–58 per flower) observed in the middle style region (Figure 3.1B). Differences between treatments were observed at the next stage of fertilisation however, with the number of ovules penetrated by pollen tubes significantly higher ($P < 0.001$) for the intraspecific CT cross (45.6 per flower) than all other cross treatments (Figure 3.2B). The number of penetrated ovules for the interspecific CCC and the three hybrid cross treatments were similar. Five weeks after fertilisation, reproductive success remained highest for the CT cross, with the number of developing embryos (47.4 per capsule) significantly higher ($P < 0.001$) than all other crosses (Figure 3.3B). The number of developing embryos per capsule also varied significantly between remaining crosses, with the interspecific *C. c. citriodora* cross significantly higher than the CT×CCV hybrid 1 and CT×CCV hybrid 2 crosses, but similar to the CT×CCC hybrid cross. Size of developing embryos, five weeks after fertilisation was significantly higher ($P < 0.001$) in the three hybrid backcross treatments, than the intraspecific CT and interspecific CCC cross treatments (Figure 3.4B).

Twelve weeks after pollination, all crosses produced seed (Figure 3.5B), with highest yields (37.5 seeds per capsule) for the *C. torelliana* cross ($P < 0.001$). The CT×CCC hybrid cross had an intermediate seed yield (24.5 seeds per capsule), which was significantly greater than both the CT×CCV hybrid 1 and CT×CCV hybrid 2 crosses. The interspecific CCC cross also had intermediate seed yields, but was not significantly different from the three hybrid backcross treatments. Capsule retention percentage at maturity was similar (11.3–22.6%) for all cross treatments and not significantly different (Table 3.2). Seed viability percentage (Table 3.2) was lowest ($P < 0.001$) for the interspecific CCC cross (68.3%), whereas the intraspecific CT and three hybrid backcrosses were uniformly high (93.9–98.3%).

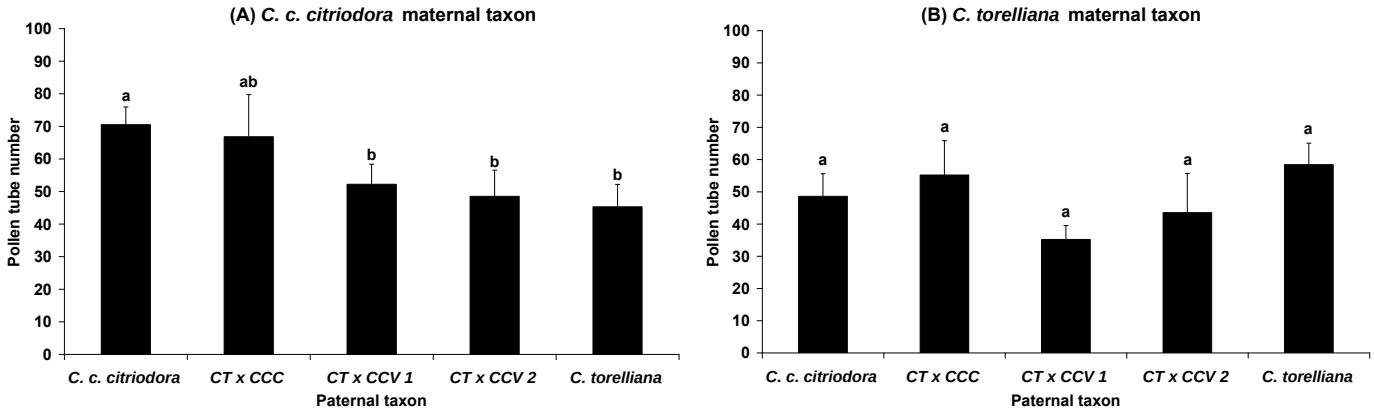

Figure 3.1. Number of pollen tubes in mid stylar region for (A) *C. c. citriodora* maternal taxon or (B) *C. torelliana* maternal taxon, crossed with five paternal taxa. Means with different letters are significantly different (P< 0.05).

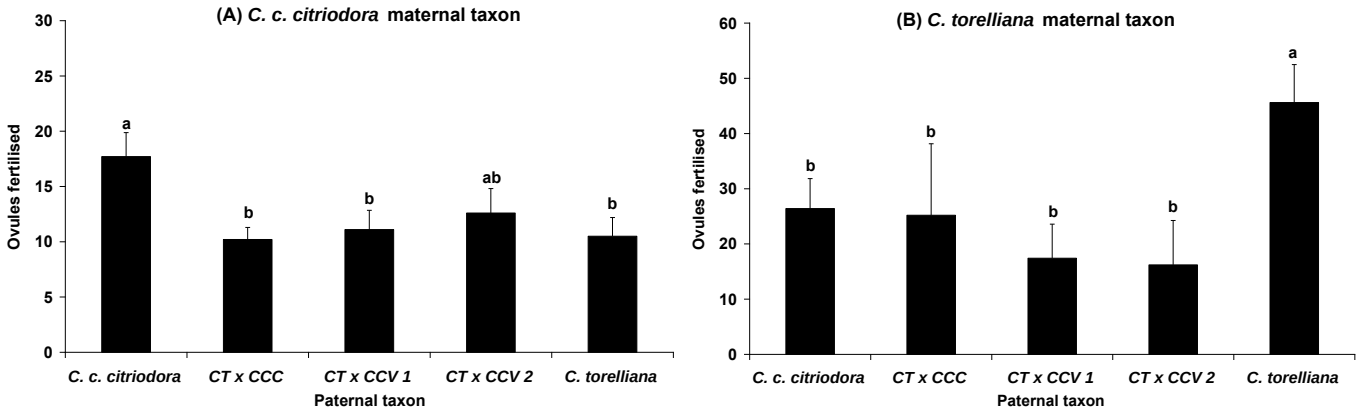

Figure 3.2. Number of fertilised ovules for (A) *C. c. citriodora* maternal taxon or (B) *C. torelliana* maternal taxon, crossed with five paternal taxa. Means with different letters are significantly different (P< 0.05).

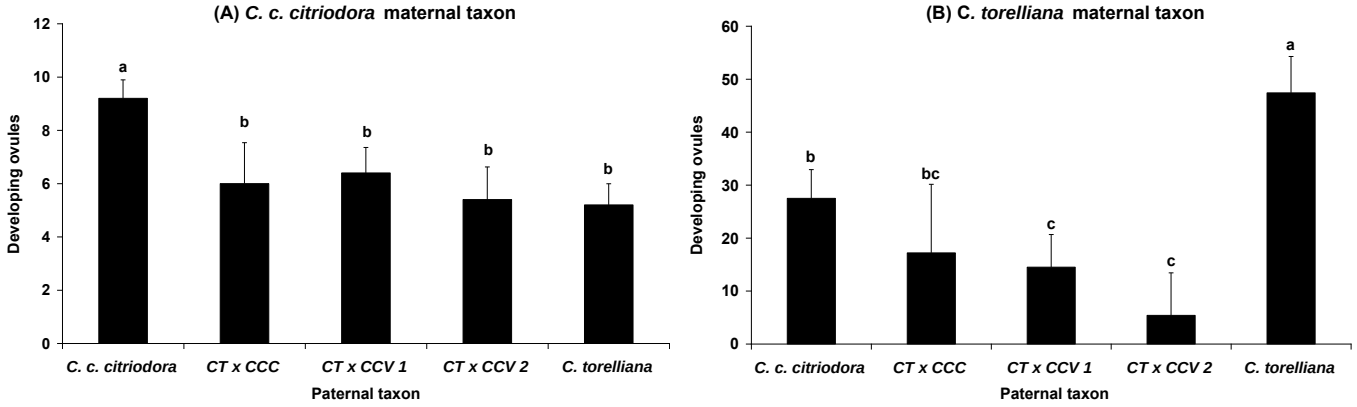

Figure 3.3. Number of developing ovules five weeks after pollination, for (A) *C. c. citriodora* maternal taxon or (B) *C. torelliana* maternal taxon, crossed with five paternal taxa. Treatment means with different letters are significantly different (P< 0.01).

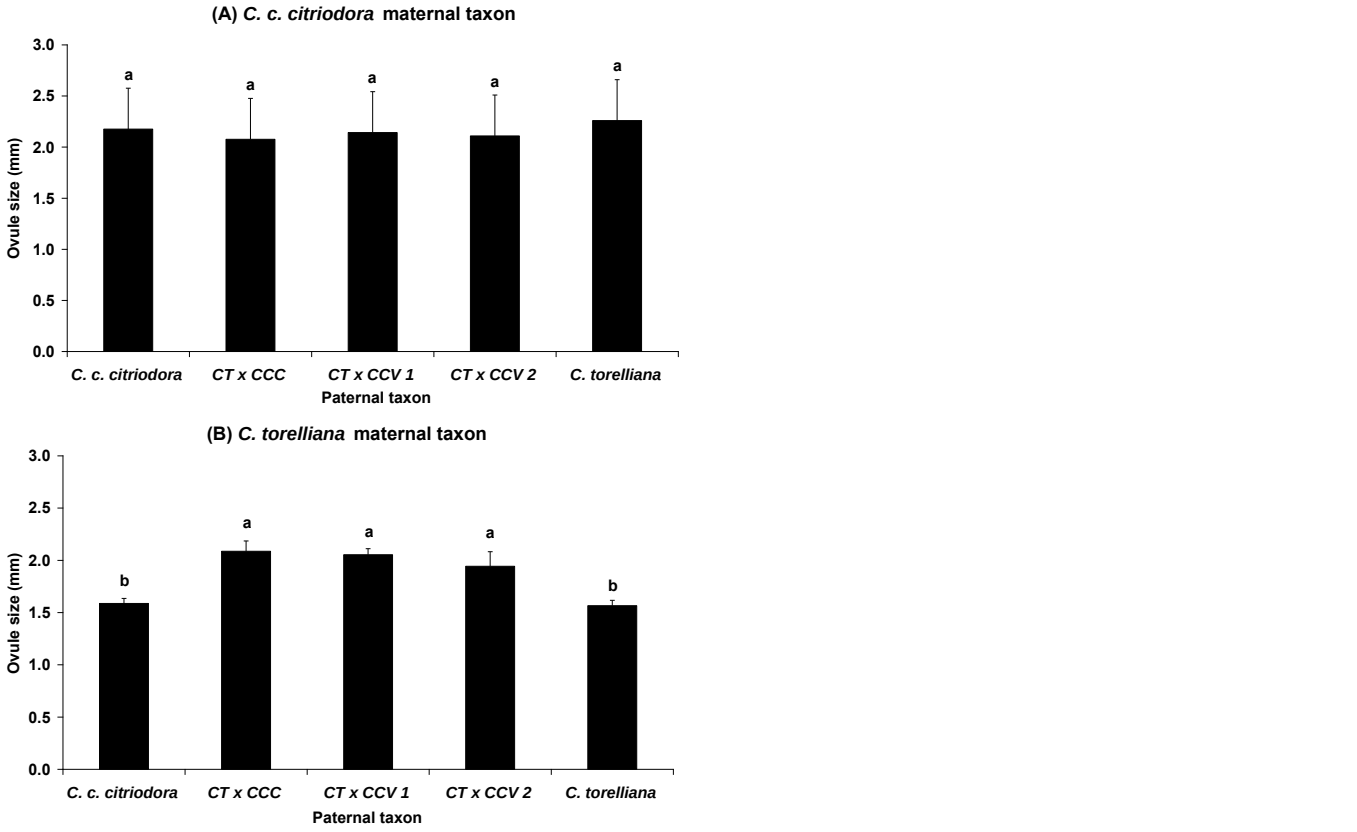

Figure 3.4. Size of developing ovules five weeks after pollination, for (A) *C. c. citriodora* maternal taxon or (B) *C. torelliana* maternal taxon, crossed with five paternal taxa. Treatment means with different letters are significantly different (P< 0.001).

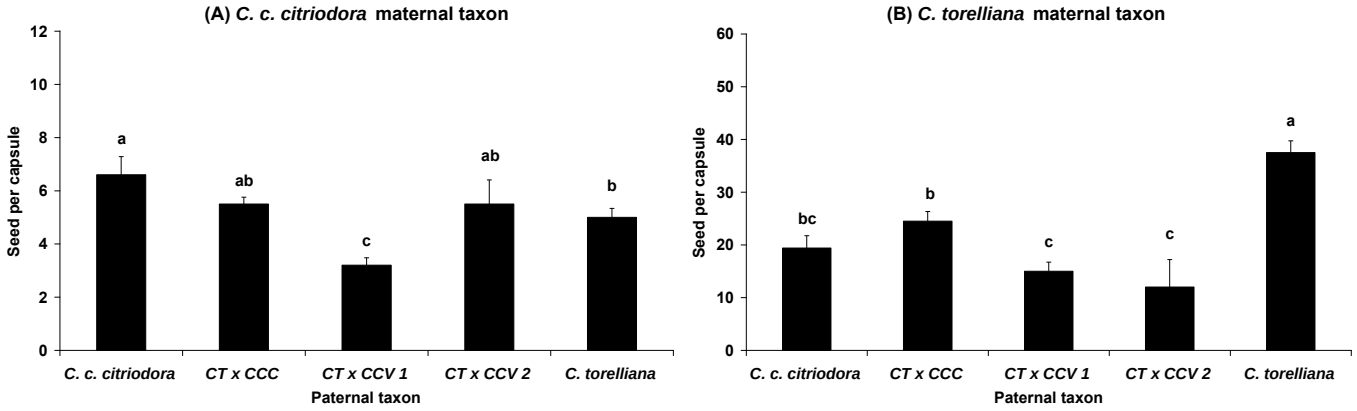

Figure 3.5. Seed number per capsule, for (A) *C. c. citriodora* maternal taxon or (B) *C. torelliana* maternal taxon, crossed with five paternal taxa. Treatment means with different letters are significantly different (P< 0.001).

Table 3.2. Capsule retention percentage at maturity and germination percentage for *C. c. citriodora* or *C. torelliana* maternal taxon, crossed with five paternal taxa. Treatment means with different letters are significantly different (P< 0.05).

	Maternal taxon			
	CCC		CT	
Paternal taxon	Capsule retention %	Germination %	Capsule retention %	Germination %
CCC	)0.3	'2.0 a	1.3	)8.3 b
CT×CCC hybrid	)4.0	)4.2 b	'2.6	)3.9 a
CT×CCV hybrid 1	'3.4	'2.3 a	7.0	)6.1 a
CT×CCV hybrid 2	)3.4	)3.5 a	2.0	-
CT	)1.5	'8.9 a	7.8	)8.3 a
P value	).746	).001	).329	: 0.001

3.1.3.3 Discussion: Gene Flow from Reciprocal and Advanced Generation Hybrids

3.1.3.3.1 Reproductive isolating mechanisms

Interspecific and advanced generation hybrids were successfully created using CCC or CT as the maternal taxon, despite activity of numerous prezygotic reproductive isolating barriers. Prezygotic isolation was observed early in the fertilisation process for the CCC maternal taxon, with lower numbers of pollen tubes in the style for the interspecific CT and both the CT×CCV hybrid 1 and CT×CCV hybrid 2 crosses. Reproductive isolation was not recorded in the *C. torelliana* maternal taxon at this same stage. Early reproductive isolation in eucalypts is recognised at numerous prezygotic stages including impeded pollen adhesion and germination on the stigma (Dickinson et al., 2012; Potts and Marsden-Smedley, 1989) and disrupted pollen tube growth through the style towards the ovaries (Dickinson et al., 2012; Ellis et al., 1991; Wallwork and Sedgley, 1995).

The next stage of fertilisation; pollen tube penetration of the ovule micropyle was an important site of reproductive isolation for both the CCC and CT maternal taxa. Almost all interspecific and advanced generation crosses had a lower number of ovules penetrated by pollen tubes than the intraspecific crosses. The cross between the CCC maternal taxon and the CT×CCV hybrid 2 paternal taxon was the only treatment with a similar number of penetrated ovules to the intraspecific cross. Pollen tube penetration of the ovule micropyle is recognised as one of the primary sites of interspecific reproductive isolation in *Corymbia,* including CT×CCC (Dickinson et al., 2012) and is a common site of reproductive isolation in other eucalypt species (Ellis et al., 1991; Sedgley and Granger, 1996; Sedgley and Smith, 1989). The identification of prezygotic stages as important locations of interspecific isolation for the CCC and CT maternal concurs with similar findings by Dickinson et al. (2012), who concluded that reproductive isolation between CT and a wider range of interspecific crosses was primarily prezygotic. In many species, reproductive isolation is generally most common at prezygotic, rather than postzygotic stages, as this conserves ovules for fertilisation by more desirable pollen and minimises wastage of plant resources via embryo abortion (Waser and Price, 1991).

Postzygotic embryo development and survival varied between the CCC *a*nd CT maternal taxa. Early differences in prezygotic reproductive success were carried through to seed maturity for the CT maternal taxon, indicating little activity of postzygotic isolation. However, the CCC maternal taxon experienced substantial decreases in embryo numbers between fertilisation (pollen tubes penetrating the micropyle) and seed maturity. Embryo abortion was high during the five week period after pollination for all cross treatments and also to maturity for the intraspecific CCC and CT×CCV hybrid 1 crosses. The large differences in early reproductive success between crosses were hence largely annulled, resulting in a similar seed number per capsule for the intraspecific CCC, CT×CCC hybrid and CT×CCV hybrid 2 crosses. Resource allocation is a major cause of postzygotic embryo degeneration and abortion in eucalypts (Sedgley and Granger, 1996; Pound et al., 2002, Suitor et al., 2008) and is the probable cause of postzygotic abortion within the *C. c. citriodora* maternal taxon.

In this study, the CCC and CT maternal taxa demonstrated different resource allocation and embryo development strategies. The CCC maternal taxon employed a conservative reproductive strategy (Moles et al., 2005), allowing only a small number of large, healthy seed to develop through to maturity, despite often high early reproductive success. Similar responses have been found in *E. globulus* (Pound et al., 2002) and *E. nitens* (Pound et al., 2003) *C. c. citriodora* populations naturally occur in marginal low rainfall environments (CPBR, 2006) hence this may be a useful survival strategy. However, the CT maternal taxon

has an opportunistic reproductive strategy, managing resource allocation and competition to allow the production of high numbers of smaller seed or small numbers of larger seed, depending on initial reproductive success. CT populations naturally occur in high rainfall environments (CPBR, 2006) hence relative seed size may have less impact on field survival. Similar results were observed by Dickinson et al., (2012) with CT×CTess and CT×*C. intermedia* hybrids producing lower numbers of embryos but of a larger size than the intraspecific CT control.

Percentage capsule retention was not influenced by cross treatment for either the CCC or CT maternal taxon. Similar results were obtained in a study by Dickinson et al., (2012), whereby interspecific crosses between the CT maternal taxon and either CCC, CCV or *C. henryi* paternal taxa, all resulted in equivalent capsule retention rates. Capsule retention in eucalypts is controlled by fertilisation level and resource competition, which justifies the commitment of valuable resources to fruit and seed production (Suitor et al., 2008). Although seed number per capsule varied between cross treatments in our study, sufficient embryo fertilisation was achieved to stimulate similar capsule development and retention through to maturity for all crosses.

Differences in seed viability percentage between crosses were measured for both maternal taxa in our study. Low seed viability was measured within the CCC maternal taxon for the CT×CCC hybrid cross and within the CT maternal taxon for the CCC cross. Inviability in hybrid progeny is caused by the deleterious interactions of genes from the same or different loci from the parental species (Levin, 1978) and is recognised as a major impediment to progress in hybrid breeding programs (Potts and Dungey, 2004). Inviability is expressed by high mortality and as abnormal phenotypes at germination, seedling development or as young trees (Barbour et al., 2006; Lopez et al., 2000). Inviability of *Corymbia* hybrids is well recognised with reduced seed viability identified for a range of interspecific crosses (Dickinson et al., 2012), and F1 and F2 families (Shepherd et al., 2006) when compared to intraspecific controls.

3.1.3.3.2 Reciprocal interspecific hybridisation

Successful reciprocal interspecific hybridisation between CCC and CT was achieved utilising either species as the maternal or paternal taxon. Structural differences in flower morphology between parent species are recognised as a primary cause of bilateral reproductive isolation (Gore et al., 1990); with the pollen tubes of small flowered species (e.g. *E. nitens)* unable to travel the full distance of the style in large flowered species (e.g. *E. globulus).* CT and CCC have relatively similar flower size (CPBR, 2006) consequently successful reciprocal

interspecific hybridisation in our study is not unexpected. Interspecific reproductive productivity however was much lower for the CCC maternal taxon (5.0 seeds per capsule) compared to the CT maternal taxon (19.4 seeds per capsule). Spotted gums are recognised for their much lower seed numbers per capsule than CT, with Lee (2007) suggesting hybrid seed yields can be up to four times higher when CT *C. torelliana* is used as the maternal taxon, rather than a spotted gum species. The higher seed productivity of CT and its propensity for more regular and prolific flowering are the primary reasons why all large-scale *Corymbia* hybrid controlled-cross pollination programs in Australia, have been conducted using CT exclusively as the maternal taxon (Dickinson et al., 2010; Lee 2007; Lee et al., 2009).

The ability to hybridise reciprocally between CCC and CT may provide opportunities for the development of new genetic combinations with desirable phenotypes. The direction of the cross can influence the genetic interactions which affect reproductive isolation and hybrid inviability (Griffin et al., 2000, Potts and Dungey, 2004) and occasionally the heritability of traits within hybrid progeny may have a bias towards the maternal parent (Assis, 2000; Delaporte et al., 2001). CCC and CCV have a number of desirable morphological characteristics which are superior to CT, including wider environmental adaptability (rainfall: 600–2000 mm / year), more desirable tree architecture (greater straightness, less branching) and greater wood quality (CPBR, 2006, Smith et al., 1991). A pragmatic rule in hybrid breeding suggested by Griffin (2000) is to use the parental species with the most desirable wood properties as the maternal taxon. Lee et al., (2007) has also identified a partial dominance of heritability within *Corymbia* hybrid progeny towards the spotted gum parent for the traits of branching and form. A reciprocal cross utilising a spotted gum species as the maternal taxon, may result in progeny with desirable wood traits more closely associated with spotted gum, which can be screened for plantation forestry potential.

3.1.3.3.3 Advanced generation hybridisation

Advanced generation backcross *Corymbia* hybrids were successfully created with both the CCC and CT maternal taxon, when backcrossed with the CT *C. torelliana*×CCC hybrid and two CT×CCV hybrid paternal taxa. This study is the first published result of controlled pollination using F1 *Corymbia* hybrid taxa, backcrossed onto either parental species. To date, only limited research has been conducted on advanced generation *Corymbia* hybrids; with variable survival and seedling performance of controlled-cross pollinated F2 families (Shepherd et al., 2006; 2008a) or spontaneous F2 and F3 families (Verma et al., 1999; Verma and Sharma, 2001).

Backcross hybrids are recognised as a highly promising form of advanced generation hybrids, as many of the reproductive isolating barriers to fertilisation have already been circumvented during production of the F1 hybrid generation (Arnold et al., 1999; Potts et al., 2000). Other forms of advanced generation eucalypt hybrids; F2's and three-way crosses are highly disposed to hybrid breakdown due to disruption of co-adapted gene complexes or loss or duplication of chromosomal segments (Potts and Dungey, 2004). In this study variation in reproductive success was identified between advanced generation hybrid crosses within both maternal taxa. The CT×CCC hybrid cross performed particularly well, whereas the CT×CCV hybrid 1 cross was the poorest performing cross for both maternal taxa. Variation in reproductive success between hybrid pollen families may be attributed to specific genetic combining interactions between individuals, known as specific hybridising ability (SHA; Nikles and Newton, 1991). SHA has resulted in variable reproductive success and field performance between F1 hybrid families of *Pinus elliottii* (Brawner, et al., 2003) and *C. torelliana* (Lee et al., 2009). Isolation-by-distance, whereby genetic distinction increases with greater geographic distance (Ochieng et al., 2010; Shepherd et al., 2008b) may also explain the often greater reproductive success of the CT×CCC hybrid cross, relative to the CT×CCV hybrid crosses. CT and CCC occur naturally in adjacent forests in north Queensland, whereas CCV occurs > 1000 km south of the nearest natural CT population.

The ability to create backcross advanced generation *Corymbia* hybrids offers new opportunities for commercial *Corymbia* hybrid breeding programs. It is widely recognised that the most optimal genotypes and greatest gains to be made from forestry hybrid breeding, will be achieved through the development of complex advanced generation hybrid combinations (Brawner et al., 2005; Griffin, 2000; Kerr et al., 2004). Individual F1 *Corymbia* hybrid families have shown great promise (Lee et al., 2009); however, there would be numerous desirable traits more closely associated with individual parental species, which could be amplified by hybrid backcrossing. Improving the amenability of *Corymbia* hybrid clones to vegetative propagation is recognised as a key priority for successful hybrid commercialisation (Hung and Trueman, 2011; Trueman and Richardson, 2008). CT has higher amenability to vegetative propagation than CCC with the F1 hybrid progeny of these parental species inheriting this trait with a bias towards the CT taxon (Assis, 2000). A backcross between CT and an F1 hybrid parent could produce advanced generation hybrid progeny with greater amenability to vegetative propagation and hence clonal deployment. Other desirable traits of CT which could be amplified by backcrossing include disease tolerance to *Q. pitereka* (Pegg et al., 2009) and browsing resistance to certain insect pests (Nahrung et al., 2011). Alternatively, the spotted gums have greater structural timber properties including density, hardness and strength (Smith et al., 1991) and tree form and branching characteristics, than

C. torelliana (Lee 2007). A backcross between CCC and an F1 hybrid parent could produce advanced generation hybrid progeny with greater wood quality.

The *Corymbia* hybrid breeding program initiated in Australia in 1999, currently utilises a Reciprocal Recurrent Selection – Selecting Forwards (RRS-SF) strategy (Nikles and Griffin, 1992), whereby the parent species are improved via recurrent selection in subpopulations, combined with concurrent testing of a wide range of hybrid progeny combinations (Lee et al., 2009). The development of a synthetic breed using advanced generation hybrid breeding is an alternative strategy. Maturing hybrid breeding programs such as the *Pinus elliottii* × *P. caribaea* hybrid program in Australia, have progressed well beyond the F1 generation, with advanced generation hybrids (including F2, F3 and backcross components) incorporated into breeding strategies to develop a multi-species synthetic breed (Brawner et al., 2005; Kerr et al., 2004). Advanced generation *Corymbia* hybrid breeding is still in its initial stages and there are concerns of poor seed yields, inviability and segregation, particularly in F2 families (Shepherd et al., 2006). The results from our study provide encouragement that advanced generation backcross hybrids and the potential development of a synthetic breed for the *Corymbia* complex may be possible for future *Corymbia* hybrid breeding.

3.1.3.3.4 Environmental risks

The creation of viable, reciprocal interspecific and advanced generation backcross *Corymbia* hybrids in this study provides useful empirical data of the likelihood of exotic gene flow from *Corymbia* plantations into sympatric native *Corymbia* populations. Results from our study were applied to a risk assessment framework for pollen-mediated gene flow from eucalypt plantations in Australia developed by Potts et al. (2003), to assess gene flow potential (Table 35). This framework incorporates the main isolating factors which influence the success of hybridisation and gene flow; environmental pre-mating barriers (geographic and ecological isolation), endogenous post-mating barriers (prezygotic isolation, postzygotic isolation) and interacting environmental/endogenous post-mating barriers (hybrid fitness).

Environmental isolating mechanisms, particularly geographic distance and flowering synchronicity are considered important drivers of natural reproductive isolation in *Corymbia* (Barbour et al., 2008). The establishment of exotic *Corymbia* plantations in close proximity to sympatric native *Corymbia* plantations, clearly circumvents the first of these key isolation mechanisms. Effective pollination distance between plantation and native populations is also likely to be large for the *Corymbia,* as their large flower size is attractive to a wide range of generalist pollinators including bats and birds (Bacles et al., 2009) which can travel in excess of 50 km in a day to access pollen sources (Southerton et al., 2004). Flower abundance

controls the quantity of exotic pollen which can be released and is another factor influencing gene flow risk. CT has substantially greater flower abundance than CCV, with the F1 hybrid intermediate between parental species (Barbour et al., 2008).

Table 3.3. Framework for assessing pollen-mediated gene flow risk from CCC, CT or CT×CCC F1 hybrid plantations into sympatric native forest populations of CCC or CT (based on Potts et al., 2003).

Plantation paternal taxon	CCC	CT	F1 Hybrid	
Native forest maternal taxon	CT	CCC	CCC	CT
Gene flow risk; pre-mating				
Pollen vector similar	High	High	High	High
Pollen vector highly mobile	High	High	High	High
Pollen (flower) abundance high	Low	High	Moderate	Moderate
Flowering season overlaps	Low-Moderate	Low-Moderate	Moderate	Moderate
Gene flow risk; post-mating				
Pre-zygotic isolation is weak	Moderate	Moderate	Moderate-High	Moderate
Postzygotic isolation is weak	High	High	Moderate-High	High
Hybrid seed yields high	Moderate	Low	Moderate	Moderate
Hybrid seed germination high	High	Moderate	Moderate-High	High
Hybrid seedling viability high	Unknown	Unknown	Unknown	Unknown
Overall risk	Moderate	Moderate	Moderate-High	Moderate

(Risk categories; Low, Low-Moderate, Moderate, Moderate-High, High)

Corymbia species have characteristic flowering seasons. In north Queensland, the flowering times of natural populations of CCC is primarily January–June and of natural populations of CT is primarily August–November (CPBR, 2006), contributing to reproductive isolation between these species. Variability in flowering times can occur between annual seasons and between populations. The cultivated CCC maternal parents used in our study flowered prolifically between July – September and natural populations of CCV are known to flower in every month of the year (CPBR, 2006). The CT×CCC hybrid and two CT×CCV hybrid paternal parents used in our study flowered prolifically between June–August. The intermediate inheritance of flowering phenology in these F1 *Corymbia* hybrids concurs with similar results for other eucalypt hybrids (Lopez et al., 2000, Verma et al., 1999). The flowering patterns observed in our study suggest that there is a moderate risk of flower synchronicity between plantations of CT, CCC and their hybrids and native *Corymbia* populations.

The results from this study indicate that pre- and postzygotic endogenous isolating barriers between crosses of CT, CCC or their hybrids are relatively weak. Reproductive isolation between species generally increases with increasing taxonomic distance between parent

species (Dickinson et al., 2012; Ellis et al., 1991; Griffin et al., 1988), with closely related species more likely to form interspecific hybrids than distantly related species. Infrageneric clades within the *Corymbia* are closely related, which is reflected in their unusually high propensity to form hybrids across taxonomic groups (Griffin et al., 1988). CT (section *Torellianae*) and spotted gum species (section *Maculatae*) have a close relationship (Hill and Johnson, 1995) and are both classified within subgenus *Blakella* (Parra-O et al., 2009). Native spotted gum populations would appear at most risk of genetic pollution as prezygotic isolation between interspecific and advanced generation hybrid crosses was either ineffective or only mildly effective. Native CT populations appear to be at a lower risk of genetic pollution, with reproductive isolating barriers resulting in some reductions in seed yield for the interspecific and advanced generation hybrid crosses examined.

In our study, hybrid viability was assessed at the germination stage, with the seed germination percentage of hybrid crosses generally equivalent to the intraspecific crosses for both maternal taxa. Lower germination rates for the backcross between the CCC maternal taxon and the CT×CCC hybrid and the cross between the CT maternal taxon and CCC were the only cases of reduced hybrid viability. Hybrid inviability at germination has been reported for interspecific (Dickinson et al., 2012) and advanced generation (Shepherd et al., 2006) *Corymbia* hybrids; however in many cases germination of eucalypt hybrid seed is generally as successful as intraspecific seed (Ellis et al., 1991; Lopez et al., 2000).

Hybrid inviability expressed by high mortality and as abnormal phenotypes is often more pronounced after germination; during seedling development or as trees (Potts and Dungey, 2004; Volker et al., 2008). Griffin et al., (2000) estimated that only 15% of planted seedlings of *E. grandis* × *E. globulus* hybrids survived as healthy plants to age two years. F1 hybrid progeny typically display poor average survival and growth when compared to outcrossed parental controls; however a small proportion of F1 hybrid families can exceed the parental performance when grown under optimum conditions (Potts and Dungey, 2004). Lee et al., (2009) describes high variability in survival (49.6–82.6%) and growth between F1 *Corymbia* hybrid families to age three years. While it is unknown how our hybrid crosses will perform under field conditions, it is likely that the advanced generation backcross hybrids will have greater hybrid viability than interspecific F1 hybrids, as many of the causes of genetic isolation have already been circumvented during the F1 phase (Arnold et al., 1999). Studies by Potts et al., (2000, 2003) indicated that growth and survival of backcross hybrids exceeded that of F1 and F2 generations, and was often similar to intraspecific controls of both parental species.

The survival chances of exotic F1 hybrid progeny regenerating within native habitats are expected to be poorer than within plantation situations, due to harsh environmental conditions, lack of adaptation to local environments and genetic incompatibilities (Barbour et al., 2006, 2010; Lopez et al., 2000). Potts and Wiltshire (1997) estimated a continuous, low background level of hybridisation of approximately 1.62%, within native forests in Tasmania, however considered it extremely rare for any of these hybrid individuals to survive and prosper. Barbour et al., (2006) also identified clear evidence of early-age selection against exotic *E. ovata* × *E. nitens* F1 hybrids in native habitats as compared to the same hybrids in a cultivated environment. The survival chances of *Corymbia* hybrids in native habitats would also appear to be very low, with few records of spontaneous hybrids in native *Corymbia* populations (Hill and Johnson, 1995), despite observations of numerous spontaneous CT × x spotted gum hybrids surviving within cultivated CT plantings (Nikles et al, 2000; Lee 2007).

The gene flow risk assessment framework developed for the reciprocal and advanced generation hybrid crosses investigated in our study (Table 3.3) suggests a moderate – moderately high risk of pollen-mediated gene flow from plantation populations into sympatric native populations. Gene flow from F1 *Corymbia* hybrid plantations into native spotted gum populations poses the highest risk. Pre-mating factors indicate a moderately high risk of pollen movement over large distances between these populations. Post-mating factors indicate reproductive pre- and postzygotic isolating barriers are weak and early hybrid fitness is relatively high. It is likely that the main factor influencing interspecific genetic pollution from *Corymbia* species and F1 hybrid plantations into native populations will be post-germination hybrid viability including survival and hybrid fitness in native habitats. Investigation of seed sowing and seedling trials in native forest areas to quantify longer term hybrid viability is recommended.

3.1.3.4 Conclusions: Gene Flow from Reciprocal and Advanced Generation Hybrids

Successful hybridisation was achieved for all interspecific and advanced generation hybrid crosses investigated in this study, reaffirming *Corymbia* as a genus with a high hybridising propensity. Reciprocal interspecific hybridisation was successfully conducted, utilising either CCC or CT as the maternal or paternal taxon, although inherent reproductive capacity and seed yields were substantially lower for the CCC maternal taxon. Advanced generation hybrids were also successfully created when either the CCC or CT maternal taxon was backcrossed with CT× CCC or CT×CCV F1 hybrid taxa. Prezygotic reproductive isolation, particularly at the stage of pollen tube penetration of the ovule micropyle was identified for all hybrid crosses with both maternal taxa. Reproductive isolation was strongest within the CT maternal taxon, with seed yields of all hybrid crosses lower than the intraspecific cross,

although capsule retention rates were similar. Reproductive isolation was less effective within the CCC maternal taxon, with two advanced generation hybrid crosses producing equivalent capsule retention rates and seed yields to the intraspecific control. Reproductive success varied between advanced generation backcross paternal taxon, with seed yields for the CT×CCC hybrid cross, higher than the CT×CCC hybrid 2 cross, within both CCC and CT maternal taxa.

The creation of viable, reciprocal interspecific and advanced generation backcross *Corymbia* hybrids has important implications for future hybrid breeding and management of gene flow risk. The successful creation of advanced generation *Corymbia* hybrids has particular breeding advantages, which can allow for the amplification of numerous desirable traits by breeding beyond the F1 hybrid generation, and potentially allowing the development of a synthetic breed for the *Corymbia* complex. The high hybridising propensity of these *Corymbia* taxa; however, poses some moderate risks of exotic gene flow from *Corymbia* plantations into sympatric native *Corymbia* populations. Gene flow from F1 *Corymbia* hybrid plantations into native spotted gum forests poses the greatest potential threat, requiring further investigation to better quantify these risks.

3.1.4 Gene flow management: Pollen flow from Corymbia hybrid plantations

3.1.4.1 Risk Areas

Hardwood plantation development in Queensland is primarily focussed on two main regions; the Wide Bay region centred on Miriam Vale and the South Burnett region centred on Kingaroy. CCV is recognised as the hardwood species with greatest forestry plantation potential in both regions (Lee et al., 2010); however, overall productivity values are low (mean 6.7 m^3/ha MAI at age six years). Low productivity rates and high risks associated with lack of knowledge of soils, nutrition, pest and disease are considered the main impediments to further hardwood plantation expansion in Queensland (Central Queensland Plantation Investment Forum, 2011). The development of *Corymbia* hybrids provides new hope for hardwood plantation establishment in central and southern Queensland, with trials across a range of sites demonstrating significantly greater productivity for the best 20% of hybrid families over *C. c. variegata* controls (Lee et al., 2008).

Expansion of *Corymbia* hybrid plantations in central and southern Queensland is likely to occur on cleared sites in close proximity to native CCC or CCV populations. CCC is widely distributed along north-eastern Australia, with the subspecies CCC extending from near Cooktown to Bundaberg and the subspecies CCV from Maryborough to northern New South

Wales (CPBR, 2006). *Corymbia* hybrid plantations may therefore pose gene flow risks to either CCV or CCV native populations in the Wide Bay region or to CCV native populations in the South Burnett region.

3.1.4.2 Risk Management

Pollen-mediated gene flow between plantation and native eucalypt species is recognised, and implementation of strategies to minimise the risk and consequences of genetic pollution is important if Australian forestry is to be considered sustainable (Potts et al., 2003). Developing a sound knowledge of the pollination and reproductive biology of the plantation and native populations is required to develop a complete framework for the assessment of gene flow risk.

A typical gene-flow risk assessment framework consists of the five key factors:

1. Pollen production and release from plantations;
2. Flowering phenology and interspecific synchrony;
3. Distance of pollen dispersal from plantations;
4. Endogenous reproductive isolating barriers; and
5. Hybrid survival and fitness.

The results from this project have greatly enhanced knowledge of the reproductive biology of *Corymbia* species and hybrids, particularly the mechanisms of endogenous reproductive isolating barriers (factor 4, above). A preliminary risk assessment framework presented in Table 3.3, based on results from our study and incorporating complimentary data from a review of *Corymbia* gene flow risk (Barbour et al., 2008), suggests a moderate – high risk of some pollen-mediated gene flow from *Corymbia* hybrid plantations into nearby CCC or CCV native populations. Similar flowering phenology, overlapping flowering patterns, similar and highly mobile pollen vectors and the limited activity of pre- and postzygotic isolating barriers between *Corymbia* hybrids and parental species, suggest some gene flow between sympatric populations is likely.

3.1.4.3 Knowledge Gaps

In order to fully quantify the risks of introgression of foreign genes from exotic *Corymbia* hybrid plantations into sympatric native *Corymbia* plantations, a number of research gaps need to be addressed. Barbour et al. (2008) recommended further research on *Corymbia* gene flow risks was required within the following nine research themes:

1. Reproductive attributes of the plantation species;

2. Flowering phenology of the native species;
3. Pollen dispersal and vector behaviour;
4. Cross-compatibility with native *Corymbia* species;
5. Levels of hybridisation at plantation boundaries;
6. Patterns of pollen dispersal from plantations;
7. Seed dispersal from *C. torelliana* F1 Hybrids;
8. Hybrid establishment and fitness; and
9. Risk assessment of the potential of gene flow.

Published studies on *Corymbia* pollination biology (Bacles et al., 2009; Barbour et al., 2008; Southerton et al., 2004) have provided some useful preliminary data on gene flow risks for the first three of these research themes. Comprehensive information on flowering phenology, synchrony and pollinator behaviour that is specific to *Corymbia* hybrids, however, is incomplete and further investigation is required to fully address these gene flow risk factors. The fourth research theme, cross-compatibility with native *Corymbia* species, has been well addressed by the outcomes from the current project. The results from this study indicate that there are few endogenous impediments to gene flow between *Corymbia* hybrid paternal trees and a CCC maternal parent, in situations where pollen is successfully applied to a receptive female flower. Some variation in reproductive success between hybrid families was observed however, indicating gene flow risks may vary for different hybrid families.

The post-seed production research themes 5-8 (above) are the main areas where little knowledge of the potential for *Corymbia* gene flow currently exists. Poor hybrid survival and fitness in native forest environments is recognised as a major controlling mechanism inhibiting gene flow between species in native forest populations (Potts et al., 2003). The survival chances of exotic F1 hybrid progeny regenerating within native habitats are expected to be poorer than within plantation situations, due to harsh environmental conditions, lack of adaptation to local environments and genetic incompatibilities (Barbour et al., 2006, 2010; Lopez et al., 2000). Potts and Wiltshire (1997) estimated a continuous, low background level of hybridisation of approximately 1.62%, within native forests in Tasmania, however considered it extremely rare for any of these hybrid individuals to survive and prosper. Barbour et al., (2006) also identified clear evidence of early-age selection against exotic *E. ovata* x *E. nitens* F1 hybrids in native habitats as compared to the same hybrids in a cultivated environment. The survival chances of *Corymbia* hybrids in native habitats would also appear to be very low, with few records of spontaneous hybrids in native *Corymbia* populations (Hill and Johnson, 1995), despite observations of numerous spontaneous CT× spotted gum hybrids surviving within cultivated CT plantings (Nikles et al, 2000; Lee 2007).

Never-the-less, hybrid progeny may occasionally emerge which have a unique combination of traits which favour their survival in a particular environmental niche.

It is recommended that an important focus of future *Corymbia* gene flow research should include the controlled establishment of seed sowing and seedling trials in native forest areas to quantify longer term hybrid viability. Collection of this information is essential to accurately quantify the actual risks of introgression of foreign genes into native *Corymbia* populations.

3.2 Potential risks of seed dispersal from plantations of *Corymbia* hybrids

3.2.1 Introduction

Corymbia torelliana has been used extensively in hybrid breeding programs in subtropical and tropical forestry in Queensland. The species occurs naturally in rainforests and rainforest margins in the wet tropics region of Far North Qld between Shipton‘s Flat, near Cooktown and Mt Fox, near Ingham. *C. torelliana* is a declared weed where it is planted in areas outside of the wet tropics. It is now listed as a noxious weed by many local councils between Grafton and Mackay and there are bans on selling, propagating and distributing the species.

C. torelliana has a unique seed dispersal syndrome that contributes to its weediness in areas where it has been introduced. Seeds are dispersed by bees, sometimes up to 1km from the parent tree. Native stingless bees build their nests from plant resins, and many species forage for resin inside the mature capsules of *C. torelliana*. When the bees forage for resin, *C. torelliana* seeds become attached to the resin droplets carried by bees. The bees eventually discard the seeds outside their nest entrances. Seeds dispersed by bees are almost all viable and germinate and establish around hives and wild nests.

Some beekeepers claim that *C. torelliana* is harmful to stingless bees. Claims are that the seed “clogs” the nest and prevents bee movement, and that the resin from *C. torelliana* tends to collapse when used in nest structure, causing the colony to die. *C. torelliana* has been banned from new plantings and actively removed by local councils from amenity plantings. *Corymbia torelliana* hybrids have great potential for sustainable plantation forestry in many areas of tropical Australia, but it is unknown whether the hybrids will have similar resin and capsule characteristics to *C. torelliana*.

In this study, we comprehensively assessed *Corymbia torelliana* x Spotted Gum (*Corymbia citriodora* subsp. *variegata, C. henryi, C. maculata*, and *C*. *citriodora* subsp. *citriodora*) in

experimental plantations for their attractiveness to native stingless bees, *Trigona carbonaria*. The aims of the study were twofold; first to determine if *Trigona* bees were attracted to *C. torelliana* hybrids and foraged for resin, a syndrome that could potentially result in subsequent dispersal of hybrid seeds. The second aim was to identify if *C. torelliana* hybrids have the capsule characteristics that allow stingless bees to successfully enter, forage for resin and subsequently disperse seed. We examined capsule length to width ratio, external and internal rim diameter, valve retraction, column collapse, resin in capsules and seed size.

3.2.2 Methods

Field observations were undertaken in three fruiting seasons (January-May) from 2009-2011. Observations took place on three *Corymbia* complex hybrid progeny trial locations in south-east Queensland, Devil Mountain (2009-2011), Coolabunia (2010) and Amamoor (2011). All *C. torelliana* and hybrids are in experimental plantations were of reproductive age.

We initially examined all fruiting trees within the plantations for mature capsules showing signs of opening. Mature capsules were deemed to be mottled green and brown. Trees were selected to be studied if they met the criteria of at least 50 mature capsules in a cluster. The selection of trees was highly dependent on the maturity of capsules at the time of selection and thus each year, the number of each hybrid cross examined varied (Table 3.4). In all cases, *Corymbia torelliana* control trees were examined. The percentage of *C. torelliana* and *C. torelliana* hybrid trees each year with mature buds was calculated for the Devil Mountain and Coolabunia site. Incomplete data sets prevented the same calculations for Amamoor and calculations included original trees planted that are now dead.

Table 3.4. Number of trees of each Spotted Gum hybrid cross and *C. torelliana* control trees sampled in this study. CT x CH = *Corymbia torelliana* x *C. henryi,* CT x CCV = *Corymbia torelliana* x *Corymbia citriodora* subsp. *variegata*, CT x CM = *Corymbia torelliana* x *C. maculata*, CT x CCC = *Corymbia torelliana* x *C. citriodora* subsp. *citriodora*, CT *Corymbia torelliana.*

Cross/Species	No. of sampled for bee visits	No. sampled for Capsule Measurements
CT x CH	10	8
CT x CCV	16	13
CT x CM	3	3
CT x CCC	2	1
CT (control)	30	16
Total	**61**	**41**

There were no local occurrences of *Trigona* bees observed at the Coolabunia site in 2010 and two *Trigona carbonaria* hives were introduced to the site. Hives were spaced at approximately 60 m apart within 200 m of fruiting trees. Capsule monitoring commenced five days later when strong bee activity was confirmed. Strong foraging activity of local *Trigona* bees were observed at the Devil Mountain and Amamoor sites and hives were not required at these sites in any year.

We examined trees for five days over the fruiting period each year. All observations took place between the hours of 10.00 and 16.00 during sunny weather conducive to bee activity. Each capsule cluster was examined for five minutes using Ziess 8x30 binoculars every two hours to assess the number of bees foraging for resin by quantifying the number of bees observed entering and exiting capsules. Bee entry was recorded if a *Trigona* bee was observed entering or exiting a capsule. The presence of seed attached to bees was noted.

We sampled 20 capsules from each tree where bee observations were undertaken (Table 3.5). We removed ripe (brown mottled) unopened capsules from *C. torelliana* study tree at the beginning of the season. Capsules were removed to eliminate the potential for interference from bees collecting resin from the capsules. For each *C. torelliana* hybrid study tree, we removed ripe, unopened capsules on the last day of bee observation monitoring. In all cases, capsules were placed in a paper bag for storage and to allow capsules to dehisce.

Once the capsules were completely dehisced (approx. 14 days), the colour and the number of valves were recorded. External length (rim to attachment of pedicel) and width were measured to determine a length to width ratio (L/W). The internal diameter of the rim was measured as this directly affects accessibility to the internal features of the capsule. Capsules were then cut vertically with a razor blade from rim to pedicel attachment to examine and measure internal features. The diameter of the internal hollow was measured and the number of seeds retained in each capsule recorded.

Retraction of the septa (separating the ovary chambers) was recorded as collapsed or present and, in 2011, we also recorded valve retraction from the internal capsule wall as present or absent. Resin inside capsules was recorded as present or absent. In 2010 and 2011, resin location was recorded under four categories: (1) resin at the split between the adjoining valves against the wall; (2) resin on the surface where the cut with the razor blade was made; (3) resin located behind the dehisced valves; or (4) resin located on the wall of the ovary chambers.

The total number of seeds was counted and weighed and average number of seeds per capsule and average seed weight calculated. The difference in number of bee visits between individual trees (family) and the cross (broad cross categories) were tested using Mann-Whitney tests. Differences in morphology and internal dimensions of capsules from different individual trees and species were tested using ANOVA, with Duncan's multiple range tests where differences were detected with ANOVA. Differences in internal structure characteristics (valve retraction, septa collapse, location of resin, seed abundance and seed weight) were tested using Kruskall Wallis (>2 categories) or Mann-Whitney tests.

3.2.3 Results

A total of 536 hybrid trees of different families and crosses was planted at the Devil Mountain site and we observed very few hybrid trees producing capsules each year. Less than 1% of all hybrid trees were observed to have produced mature capsules in 2009, which increased to 2% in 2010 and 3% in 2011. Six hundred *C. torelliana* hybrids were planted at the Coolabunia site and in 2010; 2% of the hybrids had produced mature seed capsules.

3.2.3.1 Bee Observations

Trigona bees foraged for resin on capsules of all of the *C. torelliana* trees and 16.13% of the *C. torelliana* hybrid trees observed across all three years. One *C. torelliana x C. maculata* hybrid at the Devil Mountain site received bee visits in 2010 and 2011. One *C. torelliana x C. citriodora* subsp. *variegata* hybrid at the Devil Mountain site received *Trigona* bee visits in 2011. *Trigona* bees also visited capsules on three *C. torelliana x C. citriodora* subsp. *variegata* trees at the Amamoor site. Two incidences of seed attached to the legs of *Trigona* bees exiting *C. torelliana* trees were recorded over the duration of the study. There were no bees observed transporting seed from any *C. torelliana* hybrid tree at any site in any year.

The total number of bee visits to *C. torelliana* trees in three years was 3028, significantly greater (Mann-Whitney U = 28, 637, $p<0.05$) than the combined bee visits to all *C. torelliana* hybrid trees (60 bee visits), despite a similar number of trees sampled in each category (Table 3.5).

Table 3.5. Mean number of *Trigona* bee visits in a 5 minute observation period to *C. torelliana* and *C. torelliana* hybrid trees at each site.

Hybrid Cross/Species	Devil Mountain Mean No. Bee Visits (S.E) *	Coolabunia Mean No. Bee Visits (S.E)	Amamoor Mean No. Bee Visits (S.E)	No. Trees Observed
CT x CCC[a]	0	-	-	2
CT x CH[a]	0	0	-	10
CT x CCV[a]	**0.05 (0.03)**	0	**0.27 (0.08)**	16
CT x CM[a]	**0.51 (0.14)**	-	-	3
CT[b]	**8.35 (0.55)**	**4.9 (1.22)**	**3 (0.35)**	30

3.2.3.2 Capsule Morphology

Most *C. torelliana* hybrids did not show the suite of capsule characteristics that allow bees to forage for resin and disperse seeds. Capsule length to width ratio varied significantly between all of the trees (f=42.74, p<0.01), with *C. torelliana* capsules having significantly smaller average length to width ratio (0.97). *C. torelliana* hybrids were clustered by family, though differences were minimal (1.11 for CT x CCC to 1.17 for CT x CM). There were no *C. torelliana* hybrids that exhibited length to width dimensions within the range for *C. torelliana* capsules. However, 12% of the hybrid trees observed had visits from *Trigona* bees and had capsule length to width dimensions that were closest to the *C. torelliana* range.

3.2.3.3 Internal dimensions

The diameter of the internal rim of the capsules also varied significantly between trees (f=54.99, p<0.01). *C. torelliana* trees were clustered with *C. torelliana* x *C. henryi* trees with wider internal rim dimensions (average 5.72 mm, 5.64mm respectively) than the remaining hybrid crosses (Plate 3.3a, b). Overall, 36% of all hybrid trees observed produced capsules with internal rim dimensions within the range of *C. torelliana* trees. The majority of these trees were of *C. torelliana* x *C. henryi* cross; however, 4% of all hybrid trees had visits from *Trigona* bees and had internal rim dimensions within the range for *C. torelliana*.

The diameter of the internal hollow of the capsules varied significantly between trees (f=39.02, p<0.01) with capsules from *C. torelliana* trees clustered together with wider internal hollow dimensions (average 9.71 mm) while hybrid families were interspersed throughout the remaining data set. Of all the hybrid trees observed, 64% produced capsules with internal

hollow dimensions within the same range as the *C. torelliana* trees. Only 8% of all hybrid trees had visits from *Trigona* bees and were clustered with *C. torelliana* internal hollow measurements.

Overall, 16% of the hybrid trees produced capsules within the range of *C. torelliana* dimensions for all three measurements, although none of these trees attracted *Trigona* bees. Thirty-two percent of the hybrid trees observed produced capsules within the *C. torelliana* range for two of the measurements, 4% of these trees attracted *Trigona* bees.

3.2.3.4 Internal structure

There were subtle differences in the internal capsule structure (valve retraction and septa collapse) between the trees (Plate 3.3a, b, c, d). When trees were grouped according to species cross, there were some significant differences. Capsules from all *C. torelliana* trees exhibited some degree of valve retraction while capsules from 40% of hybrid trees exhibited some degree of valve retraction (Mann-Whitney $U=87.5$, $p<0.05$) although none of these hybrids attracted *Trigona* bees. Similarly, capsules from all *C. torelliana* trees measured exhibited some degree of septa collapse, while capsules from 32% of hybrid trees exhibited partial septa collapse (Mann-Whitney $U=34.5$, $p<0.05$) and 8% of all hybrids attracted bees. Only 8% of hybrids trees had capsules with a collapsed septa and retracted valves similar to *C. torelliana* trees.

All hybrids trees produced resin (Plate 3.3b, c, d) in the capsules and the location of resin was similar to that of *C. torelliana* trees with no significant differences between the trees. When grouped, trees from the *C. torelliana* x *C. henryi* and the *C. torelliana* x *C. citriodora* ssp. *variegata* crosses produced significantly fewer capsules displaying resin behind retracted valves (15-20% cf. 80-100%; Kruskall Wallis $R= 9.69$, $p<0.05$). Otherwise, there were no significant differences in resin location between categories.

C. torelliana trees produced significantly more seed (Kruskall Wallis $R=15.61$, $p<0.05$) than *C. torelliana* hybrid trees. In some cases, *C. torelliana* trees produced between 3 and 10 times the amount of seed compared to some hybrid crosses (Table 3.6). Average seed weights varied and were significantly heavier in hybrid trees (Kruskall Wallis $R=23.65$, $p<0.05$). Seed from *C. torelliana* x *C. maculata* hybrids were heavier than all other trees (Table 3.6). Average seed weight of hybrid trees that attracted *Trigona* bees was 4.00 mg (SE=0.56), significantly heavier than that of *C. torelliana* trees (1.79 mg, SE=0.1; Mann-Whitney $U=44.00$, $p<0.05$).

Table 3.6. Mean number of seed produced per capsule and average seed weight in this study. CT x CH = *Corymbia torelliana* x *C. henryi,* CT x CCV = *Corymbia torelliana* x *Corymbia citriodora* subsp. *variegata*, CT x CM = *Corymbia torelliana* x *C. maculata*, CT x CCC = *Corymbia torelliana* x *C. citriodora* subsp. *citriodora*, CT *Corymbia torelliana.*

Hybrid Cross	Avg. No. Seeds/Capsule (S.E)	Average Seed Weight (mg)	No. sampled
CT x CH	-	3.23 (0.17)	8
CT x CCV	3.86 (0.79)	3.29 (0.23)	13
CT x CM	2.70 (0.0)	5.43 (0.59)	3
CT x CCC	10.65 (0.0)	3.32 (0)	1
CT	33.58 (4.7) ***	1.79 (0.10)	16
Total			**41**

3.2.4 Discussion

No *C. torelliana* hybrids were dispersed by bees in this study. However 16% of the *C. torelliana* hybrid trees examined produced capsules that attracted *Trigona* bees and all of the *C. torelliana* trees attracted bees. Stingless bees are attracted by chemical signals and it is likely that most hybrids lack the appropriate resin chemistry to attract bees. Resin was present in all hybrid capsules. *C. torelliana* has a suite of internal and external capsule characters that enable bee dispersal. Only 12% of the hybrid trees observed produced capsules with the appropriate external dimensions (length to width ratio, internal rim and internal hollow) measurements to enable bee dispersal. None of the hybrid trees that attracted *Trigona* bees possessed the appropriate capsule dimensions. Overall, 8% all hybrids had the appropriate internal capsule structure that would allow bee dispersal. These capsules had a collapsed column and retracted valves but did not attract *Trigona* bees. Seed from the hybrid trees that attracted bees was heavier than that of *C. torelliana*, although *Trigona* bees have been known to transport up to four *C. torelliana* seed at a time.

3.2.4.1 Risks of harm to bees, hybrid seed dispersal, and weediness

We found 16% of hybrids were attractive to bees and all hybrids contained resin in their capsules. These hybrids could potentially produce resin that is collected by bees. The resin from *C torelliana* and hybrids has not been conclusively shown to cause harm to bees in any study, but this is an area that warrants further investigation. In this study, no hybrid was dispersed by bees. No hybrid was found with the complete set of characteristics that would enable bee dispersal. However, if hybrids are created from seed and deployed in large scale plantations, it is likely that a small proportion of the hybrids will inherit the complete set of

characters that enable bee dispersal from their *C. torelliana* parent. Based on our observations, we estimate that 1.5 trees in every 1000 that produce capsules will inherit these characters and have the potential to become weeds. This equates to approximately one tree per hectare if trees are planted at a density of 800 stems per ha. Therefore, large scale plantings of *C. torelliana* could contain a small proportion of trees that pose a risk of becoming an invasive weed.

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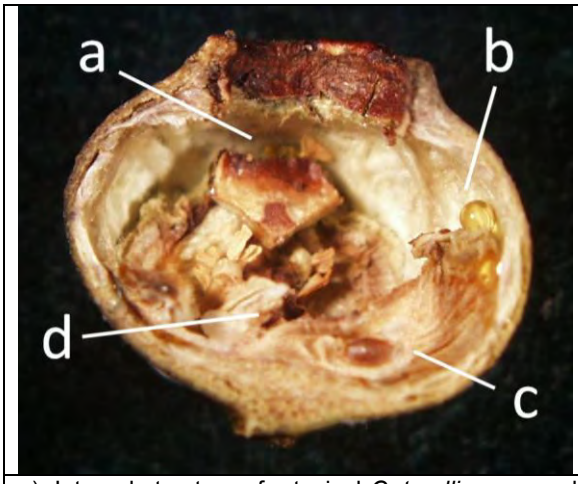
<td> 
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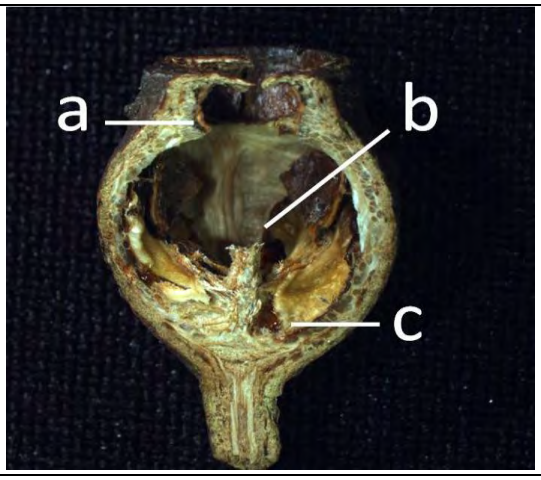
<td> 
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<td>a): Internal structure of a typical C. torelliana capsule (a) full retraction of valve from capsule wall; (b) resin behind retracted valves; (c) resin on wall of ovary chamber; (d) collapsed septa.</td>
<td>b): Internal structure of a C. torelliana x C. maculata capsule (a) smaller internal rim diameter than C. torelliana; (b) partial retraction of septa; (c) resin in split between ovary chambers.</td>
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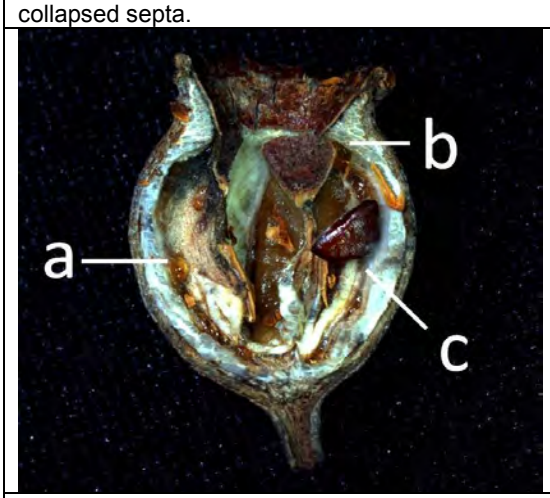
<td> 
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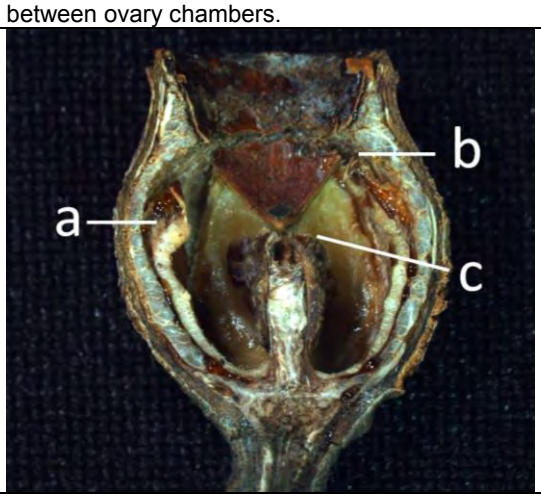
<td> 
</td>
</tr>
<tr>
<td>c): Internal structure of a C. torelliana x C. citriodora ssp. variegata capsule; (a) abundance of resin on wall of ovary chamber; (b) incomplete valve retraction; (c) large seed.</td>
<td>d): Internal structure of a C. torelliana x C. maculata capsule (a) abundance of resin behind valve; (b) incomplete valve retraction; (c) incomplete retraction of septa and absence of internal hollow.</td>
</tr>
</table>

Plate 3.3. Fluorescent microscope images; (A) germinating pollen on the stigma surface, CT×CCC, scale bar = 100 µm, (B) pollen tubes in mid stylar region, CT×CCC, scale bar = 200 µm and (C) pollen tube penetration of the ovule micropyle, CT×CT scale bar = 100 µm. Light microscope image; (D) developing embryos within the locule at age five weeks, CT× and CT×CTess (right), scale bar = 2000 µm.

3.2.5 Gene flow management: Seed dispersal from Corymbia hybrid plantations

These studies clearly show that there is a risk of gene flow from *Corymbia* hybrids. The main focus of new plantation development for *Corymbia* hybrids is likely to be central and southern Queensland. Plantations of *Corymbia* hybrids are likely to occur in close proximity to native populations of spotted gums, but less likely to occur in North Queensland in the range of native *C. torelliana* populations. As a result the major risk of gene flow is from *Corymbia* hybrid plantations to spotted gum populations.

These results clearly show that *Corymbia* hybrid pollen often produced viable seed when crossed onto spotted gum females. Therefore risks of pollen flow from *Corymbia* hybrids to spotted gums also need to be managed. There is an unknown risk of harm to native bees and a small risk of hybrids becoming weeds due to seed dispersal of hybrids by bees. *C. torelliana* is already an invasive weed in coastal native forests in southern Queensland and northern New South Wales and can no longer be contained. *C torelliana* in native forest may facilitate pollen flow and extensive, wide hybridisation within *Corymbia* species, especially between spotted gums and *C. torelliana* hybrids in plantations.

3.2.5.1 Mitigation measures

Mitigation measures have not been used yet in tropical and subtropical eucalypt species, and there is much to be learnt from the experience in temperate plantation forestry. Mitigation measures to prevent gene flow have been utilized in southern states, especially where there are high risks of pollen flow swamping of rare species close to plantations. Certification schemes such as Forest Stewardship Council and AFS are currently being revised and likely to require that risks of gene flow from plantations are assessed and mitigated against.

Mitigation measures include:

- Conduct a full risk assessment when selecting sites to identify of any potential risks of gene flow. Weed and gene flow assessments are carried out as standard practice for many forestry species proposed for deployment in southern states but this is not the case in the tropics and subtropics. A risk management analysis implemented as early as possible may help to avoid poor choices of species and circumvent gene flow problems.

- There may be an opportunity to select sites (at some distance from spotted gum forests) and hybrids that pose lower risks. Some *Corymbia* hybrids, for example *C.*

torelliana × *C. maculata*, may pose less risk of gene flow than others due to lower fertility – these could be preferentially selected for plantations.

- Set up monitoring plots for hybridisation between CT hybrids and spotted gum; i.e. seed is collected on a yearly basis and checked for hybrids. This is common practice in southern states (e.g. gene flow from *E. nitens* plantations to sympatric *E. ovata* forests). This may require long term monitoring.

- Remove wildings and *C. torelliana* from native forests near plantations. This is initially a weed issue but, in the longer term, they could facilitate gene flow.

- Screening hybrid plantations for attractiveness to bees. The incidence is likely to be low, but the potential problems are weediness and harm to bees. Seed dispersal by bees is a special case because stingless bees move the seeds several kilometres. There may be screening tools available such as chemical assessment of hybrids to determine their attractiveness to bees.

- Select and clonally propagate sterile trees. Only a small proportion of the hybrids in this study produced fruit, but this is very early in the life of the plantations. If superior sterile hybrids could be identified, they could be clonally propagated for deployment. Clonal propagation methods for *Corymbia* hybrids are well advanced. There may be advantages, for example, that the trees will not waste energy on flower and fruit production, and thereby produce higher yields of timber.

3.2.5.2 Knowledge gaps

Gene flow research is in it is infancy in plantations in the tropics and subtropics unlike in southern states where extensive investigation has been conducted over the past 15 years. There are a range of knowledge gaps that need to be filled to manage the risks of gene flow from plantations:

- How far does pollen travel in subtropical eucalypts? There is some data on gene flow in southern eucalypts. While most pollen is deposited close (within 250m) to the source, there are long tails to the distributions and commonly dispersal is found up to 1-2 km away. In subtropical and tropical *Corymbia* species the pollinators are insects, birds and bats. Vertebrate pollinators can travel in excess of 50 km in one day resulting in very long distance dispersal. However, pollen dispersal curves in tropical species are not characterised like some other eucalypts. Research would need to

focus on known corridors for bat and bird migration. This will require both genetic and morphological markers of hybridisation.

- How well do hybrids survive in the native forests where conditions may be much less favourable than plantations? This is a key knowledge gap and may show that the chance of survival is very low.

- Do hybrids produce viable see with more distant crosses within Corymbia?

- Does *C. torelliana* and hybrid resin harm stingless bees?

- Can buffer zones reduce gene flow? Buffers may reduce light levels in plantations and thus prevent flowering, or satiate pollinators to prevent long distance dispersal. However, if buffers do not share the flowering times of plantations, they may not mitigate gene flow at all. *Corymbia* hybrids tend to have a set flowering time, whereas spotted gums spotted gums can flower throughout the year – more research is needed to understand the control of flowering.

To date, there has been no evidence of hybrid recruitment into native forests but there has been no comprehensive study on this. Spontaneous hybrids frequently occur in wind breaks, from spotted gum pollen going on to *C. torelliana* but spontaneous hybrids have not yet been reported from *C. torelliana* pollen onto spotted gums. Invasiveness and genetic pollution of *C. torelliana* have already occurred in some locations in subtropical Queensland and northern New South Wales. There remains a window of opportunity to manage and contain gene flow from *Corymbia* hybrid plantations into sympatric natural *Corymbia* populations within these same areas if appropriate management actions can be developed.

Chapter 4. Benefits of the project

4.1 Benefits to Queensland

4.1.1 Contributions to the aims of the Plantation Hardwoods Research Fund

This project has successfully supported all four aims of the Plantation Hardwoods Research Fund:

1. *Support the development of a viable plantation based industry for Queensland in-line with the aims of the Statewide Forests Process*.

The identification of 108 elite varieties of *Corymbia* hybrids, *E. argophloia* and *E. cloeziana* project will provide renewed investor confidence for establishing hardwood plantations in Queensland. The project is providing the hardwood plantation industry with varieties known to possess superior wood properties, growth and form. Development of a hardwood plantation industry using these elite varieties also aligns with the aims of the Statewide Forests Process by providing certainty to the timber industry that the transition to plantations is based on tree varieties with known superior attributes.

2. *Support the development of fast growing hardwood plantations suitable for a variety of solid wood products (both sawn and non-sawn) that capitalise on the unique features of the resource*.

A core aim of the project was to characterise the wood properties of trees with elite growth and form that were identified in existing base populations of *Corymbia* and *Eucalyptus*, many of which were planted as part of the Hardwoods Queensland project. Resource characterisation has identified that plantation-grown *Corymbia* hybrids, *E. argophloia*, *E. cloeziana* and *E. pellita* are all highly suitable sources of timber for a variety of solid wood products. As a comparison, the most common species for the manufacture of structural plywood in Australia is radiata pine. On average it has a veneer MoE of about 10,400 MPa (combining inner and outer veneer samples). The average MoE results on the hardwood species in this project were superior to those of radiata pine. This is an important and valuable attribute for the Queensland plantation hardwood resource, as the resulting ply or timber from all four hardwood trees will be stronger, for the same piece size, than ply or timber derived solely from *Pinus* species.

3. *Support innovative processing and manufacturing based on a plantation hardwood resource to produce outputs including both solid wood and composites*.

Breeding populations of eucalypts in Queensland, such as those established by Hardwoods Queensland, have reached the age where selection for wood properties is possible. This project has produced a critical resource for efficient high-throughput processing and manufacturing, in the form of hardwood tree varieties that provide a known and consistent product for solid wood or composites. As an example, the four hardwood taxa evaluated for wood properties all showed great promise for veneer production. The *Corymbia* hybrids had a mean basic density of 614 kg/m^3 compared to approximately 800 kg/m^3 for plantation / native forest-derived trees. This is equivalent to 77% of native forest-derived trees at approximately 8 years old. Similar-aged *E. cloeziana* had 73% of its native forest basic density. The other two taxa, *E. argophloia* and *E. pellita*, were evaluated at age 13 years. These two species had 85% and 72% of their respective mature native forest basic densities. The available published data indicates that further increases in basic density to reach mature values are important, and it is reasonable to suggest that this may be reached around the age of 25–30 years, which is close to the final harvest age for a sawlog management regime.

4. *Facilitate the transition from native forest logging to a plantation based industry and to support industry adjustment through the transition*.

An impediment to rapid establishment of a plantation based industry in Queensland was a lack of investor confidence in the unimproved tree germplasm that was previously available for subtropical and tropical plantations. This project has allowed the release of new hardwood tree varieties that are expected, with confidence, to provide superior wood quality, growth and form. The 60 *Corymbia* hybrid clones released through this project will have significantly increased growth, disease resistance, form, heartwood percentage and straightness, with increased performance in the order of 30% over that of plantations established using wild *Corymbia* species seed. Plantations established using seed from the 24 *E. argophloia* and 24 *E. cloeziana* varieties selected in this project will have increased performance in the order of 20% due to better growth, straightness and form, and increased heartwood percentage over plantations established using wild seed.

4.1.2 Contributions to Queensland's R&D priorities

This project has contributed strongly to three of Queensland's research and development priorities: **Environmentally Sustainable Queensland**, **Smart Industries** and **Tropical Opportunities**. The project has provided the knowledge, tools and technologies required to:

- Protect and restore Queensland's diverse ecosystems;
- Enhance productivity and create new value-adding products and services in Queensland's food and fibre industries; and
- Create globally competitive tropical expertise industries.

This project has helped to protect Queensland's native forests by assisting in the transition from native forest logging to the establishment of sustainable hardwood plantations. The project also greatly enhanced our knowledge of the reproductive biology of *Corymbia* species and hybrids, which is critically important for managing the potential risks of gene flow from hardwood plantations into native forests. A risk assessment framework was developed for managing gene flow from *Corymbia* hybrid plantations into native forests. This report discussed possible mitigation measures for minimising pollen-mediated gene flow from *Corymbia* hybrid plantations, and it described the risk that a very small proportion of *Corymbia* hybrids will potentially have their seeds dispersed by native bees. However, we estimate that only about 1.5 trees in every 1000 will have these seed dispersal characteristics, which equates to approximately one tree per hectare. Possible mitigation measures against gene flow include:

- Conducting risk assessments when selecting sites to identify of any potential risks of gene flow;
- Choosing sites at some distance from spotted gum forests;
- Selecting hybrids that pose lower risks of gene flow;
- Setting up monitoring plots for hybridisation between *Corymbia* hybrids and spotted gum;
- Removing wildings and *C. torelliana* from native forests near plantations.
- Screening hybrid plantations for attractiveness to bees.
- Selecting and propagating sterile trees.

This project also created knowledge and tools to enhance productivity and create new products from Queensland's hardwood plantation estate. The 60 *Corymbia* clones released from this project will have significantly increased performance in the order of 30% over that of plantations established using wild *Corymbia* species seed, and seed from the 48 *E.*

argophloia and *E. cloeziana* varieties selected in this project will have increased performance in the order of 20% over plantations established using wild seed. All of the eucalypts assessed in this project were found to have highly suitable timber for a range of both sawn and non-sawn wood products. All showed great promise for veneer production. Further, the project has shown that small-diameter trees can be used for composite products, allowing for early-age economic returns on investment from plantations. This had been a major impediment to expansion of a plantation estate that was previously focussed solely on solid wood products.

Knowledge about the wood properties and the variation of these traits across the four project taxa, *Corymbia* hybrids, *E. argophloia*, *E. cloeziana* and *E. pellita*, has also provided vital information to the Queensland Hardwood Tree Improvement Team about what wood property traits should be focussed on to maximise product value and profit for the plantation industry. As most trees were above minimum thresholds for MoE, density and radial and tangential shrinkage, the wood property traits to be focussed on in future breeding and selection efforts are heartwood percentage (for all four taxa) and wood colour (for *E. pellita*).

This project also developed expertise in the propagation of Queensland's highly valuable tropical and subtropical eucalypt varieties. Eucalypts suited to drier regions, such as the *Corymbia* hybrids, *E. argophloia* and *E. cloeziana*, have been considered internationally as very difficult to propagate. This project developed new techniques and made recommendations for propagating *Corymbia* hybrid cuttings, which increase plant production by more than 200% over alternative methods. The project also demonstrated that elite trees of *E. argophloia* and *E. cloeziana* can be captured into production nurseries using grafting methods. Queensland has a major competitive advantage in hardwood plantation forestry, being the custodian of a unique diversity of highly-desired tropical and subtropical eucalypt species. These propagation technologies place Queensland at the forefront of global efforts to mass-propagate tropical eucalypt trees. These techniques are already being used by the forest nursery industry to provide the best-possible trees for further establishment of the hardwood plantation estate in Queensland.

4.1.3 Contributions to the future development of, and collaboration between, the recipients and the project partners.

Collaboration on the project between the recipient, the University of the Sunshine Coast, and the partners, CSIRO, DEEDI, Forestry Plantations Queensland and Elders Forestry, has led to on-going collaborations and project applications between these, and other, organisations involved in forestry research in Queensland.

A crowning achievement has been the recent award of a $5.45 million grant over the next three years from the Commonwealth Government's Collaborative Research Network scheme. This grant will consolidate research projects involving forestry, water, sustainability and aquaculture between the University of the Sunshine Coast, Griffith University and the University of Tasmania. USC has already appointed five new research fellows in forestry under this scheme and is purchasing high-tech laboratory equipment to support its strategic initiatives in forestry research. The Queensland community will soon see these research outcomes in practice as the projects advance the capabilities of the forest industry, highly qualified staff are attracted to USC, and local graduates have expanded opportunities for research scholarships and mentoring within successful forestry research teams.

The collaborations between the recipient and the partners on this project have also culminated in a joint glasshouse facility being constructed at USC (approximate value $750,000). The project partners (USC, DEEDI and CSIRO) are now occupying this facility, and further collaboration between the partners is planned around this facility in areas such as tree improvement, propagation and nutrition.

USC has also partnered with each of the other project participants (CSIRO, DEEDI, Forestry Plantations Queensland and Elders Forestry) to seek funding from other state and federal schemes to support forestry research in Queensland. Funding has been sought for topics as diverse as tree improvement, myrtle rust screening, Quambalaria screening, weed management, tree nutrition, tree propagation and carbon sequestration. Many submissions are still under review, but some of the recent funding successes include:

- A research fellowship funded by the Commonwealth Department of Education, Employment and Workplace Relations to investigate clonal propagation methods for *E. cloeziana* and *E. dunnii* trees [USC, Bose Institute (India)].
- A project funded by the Commonwealth Department of Agriculture, Fisheries and Forestry to determine adaption and carbon sequestration by subtropical hardwood plantations [USC, DEEDI, CSIRO].
- A project funded by the Rural Industries Research and Development Corporation evaluating the potential for a Subtropical Tree Improvement Alliance [CSIRO, DEEDI, USC, Forests NSW].
- A sabbatical fellowship for one of the world's leading experts in eucalypt propagation, Dr Ivar Wendling, to visit Queensland for 12 months to share his knowledge and conduct research on methods for propagating *Corymbia* trees [USC, Embrapa Florestas (Brazil)].

4.1.4 Other benefits to Queensland.

This project has also delivered on the Queensland Government's *Towards Q2* targets of:

- **A strong economy.** *(1) Infrastructure and growth.* We have built on Queensland's natural strengths in tropical forestry by selecting the state's best eucalypt trees to establish our future hardwood plantations. *(2) Business innovation.* We are working with local forest growers and plant nurseries to incorporate new and innovative technologies into their commercial plant production.
- **Green environment.** *(1) Reducing our carbon footprint.* Planting trees of the 108 elite varieties selected by this project will greatly increase the amount of carbon stored in Queensland's plantations and forest products. *(2) Protecting our natural landscape.* Attracting investment in plantations will underpin the state's transition from native forest logging to sustainable plantations, and minimising gene flow from hardwood plantations will maintain the genetic integrity of Queensland's valuable native forests.
- **Smart education.** *(1) Training and qualifications.* The University of the Sunshine Coast has created a teaching/research nexus in forestry, environmental science and biotechnology. Students have been undertaking placements, attending field excursions, and conducting forestry research with the project partners, including DEEDI and Forestry Plantations Queensland. USC has also developed a new third-year course, in collaboration with researchers from DEEDI, in the area of 'Forests, Carbon and Climate'. This course provides a point-of-entry to final-year students into an Honours program in forestry research.
- **Fair communities.** *(1) Disadvantage affects us all.* Employment creation in the plantation industry is centred on target regions with high levels of unemployment: Far North Queensland, Wide Bay/Burnett and the Sunshine Coast. The project has progressed in conjunction with the major plantation companies to provide confidence for investment and create jobs in these three target regions.

The project has helped develop Queensland's competitive advantage and will help develop Queensland's diverse regions by demonstrating the superior qualities of many of our native timber species. This project has shown that the project species can be grown under plantation conditions and produce similar timber to that produced by native forests. Further, the project has shown that small diameter trees can be used in composite products, allowing for early-age economic returns on investment from plantations. This was a major impediment to expansion of a plantation estate that was previously focussed solely on solid wood products.

4.2 Publications arising from the project

1. Lee DJ, Huth JR, Brawner J, Dickinson GR. 2009. Comparative performance of Corymbia hybrids and parental species in subtropical Queensland and implications for breeding and deployment. *Silvae Genetica* 58: 205–212.
2. Dickinson GR, Wallace HM, Lee DJ. 2010. Controlled pollination methods for creating *Corymbia* hybrids. *Silvae Genetica* 59: 233–241.
3. Leonhardt S, Wallace HM, Schmitt T. 2011. The cuticular profiles of Australian stingless bees mirror the unusual resin of their resin source (*Corymbia torelliana*). *Austral Ecology* 36: 537–543.
4. Lee DJ, Zbonak A, McGavin R. 2011. Development of *E. argophloia* in Queensland: lessons learnt. In: *Developing a Eucalypt Resource: learning from Australia and elsewhere* (ed. Walker J). University of Canterbury, Blenheim, New Zealand. pp. 55–66.
5. Harding KJ, Zbonak A, Lee DJ, Brown T, Innes T, Davies MP, Copley TR. 2011. Producing elite tree for high value sawlogs from the tropics. *Australian Forest Grower* 33: 30–31.
6. Dickinson GR, Lee DJ, Wallace HM. 2012. The influence of pre- and post-zygotic barriers on hybridisation between *Corymbia* sections. *Annals of Botany* (in press).
7. Trueman SJ, McMahon TV, Bristow M. 2012. Production of cuttings in response to stock plant temperature in the subtropical eucalypts, *Corymbia citriodora* and *Eucalyptus dunnii*. *New Forests* (in press).
8. Dickinson GR, Wallace HM, Lee DJ. 2012. Reciprocal and advanced generation hybrids between *Corymbia citriodora* and *C. torelliana*: Breeding opportunities and risk of gene flow. *Forest Ecology and Management* (submitted).
9. Trueman SJ, McMahon TV, Bristow M. 2012. Production of cuttings of *Eucalyptus cloeziana* in response to stock plant temperature. *Journal of Tropical Forest Science* (submitted).

4.3 Presentations

1. Lee D, Huth J, Zbonak A, Brawner J, Dickinson G, Harding K. 2012. Characterising the wood properties of tropical and subtropical timber species. Tropical and Subtropical Forestry Forum. University of the Sunshine Coast, Sippy Downs, Queensland, 17 February 2012.
2. Trueman S, Warburton P, Wendling I. 2012. Propagation research. Tropical and Subtropical Forestry Forum. University of the Sunshine Coast, Sippy Downs, Queensland, 17 February 2012.

3. Dickinson G, Wallace HM, Lee DJ. 2012. *Corymbia* pollen-mediated gene flow. Tropical and Subtropical Forestry Forum. University of the Sunshine Coast, Sippy Downs, Queensland, 17 February 2012.
4. Wallace H, Lee D, Simmons L. 2012. Risks of genetic pollution by seed dispersal - Corymbia torelliana and hybrids. Tropical and Subtropical Forestry Forum. University of the Sunshine Coast, Sippy Downs, Queensland, 17 February 2012.
5. Dickinson GR, Lee DJ, Wallace HM. 2012. Gene flow risks for commercial *Corymbia* species and hybrids. CRC for Forestry – 2012 Annual Science Meeting, Mooloolaba, Queensland, 5–6 March 2012.

4.4 Expenditure

The project expenditure by the Recipient and Partners has been consistent with the project proposal and the details provided in the project's four progress reports. The Recipient will have no unspent funds when the final milestone payments have been made to the Partners (CSIRO and Agri-Science Queensland).

PROJECT INVESTMENT SUMMARY (excl. GST)

Source	Cash ($)	Other Eligible Costs ($)	Other Ineligible Costs ($)	Total ($)
Plantation Hardwoods Research Fund	$875,000	-	-	$875,000
University of the Sunshine Coast	-	$225,260	$167,857	$393,117
CSIRO	-	$225,252	$126,289	$351,541
Agri-Science Queensland	-	$274,853	$204,505	$479,358
Elders Forestry	$65,625	-	$40,000	$105,625
Forestry Plantations Queensland Ltd	$32,812	-	$20,000	$52,812
TOTAL	**$973,437**	**$725,365**	**$558,651**	**$2,257,453**

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Appendices

Appendix 1. Location of trials

Appendix 2. Site and metrological details for trials

E. argophloia

Site description

	Expt 460a HWD	**Expt 460b HWD**	**Expt 460e HWD**
Trial type	Progeny trial	Progeny trial	Seed orchard
Location	Dunmore State Forest Office	DEEDI Dalby Agricultural College 'Glengarry', via Dalby	Dunmore State Forest Office
Latitude/longitude	27.57°S 151.08°E	27.25°S 151.36486°E	27.57°S 151.08°E
Rainfall	Dunmore Forestry Office Mean 701 mm Median 707 mm 69 years 1940–2009 (1951 missing)	Victory Downs Mean 614 mm Median 585 mm 46 years 1959–2009 (five years missing)	Dunmore Forestry Office Mean 701 mm Median 707 mm 69 years 1940–2009 (1951 missing)
Rainfall site	0.4 km south of trial site	1.1 km north-east of trial site	0.4 km south of trial site
Elevation	411 m a.s.l.	345 m a.s.l.	411 m a.s.l.
Soil type	Brown Dermosol	Grey Vertisol	Grey Chromosol
Date planted	9 April 1997	10 April 1997	9 April 1997
Spacing	5.0 m × 2.0 m = 1000 spha	5.0 m × 2.0 m = 1000 spha	5.0 m × 2.0 m = 100 spha
Thinning history	Thinned August 2002 (age 5.5 years) to 333 spha (retain one trees in each group of three)	Thinned August 2002 (age 5.5 years) to 333 spha (retain one trees in each group of three)	Thinned July 2002 (age 5.2 years) to 333 spha retaining the best tree in each group of three. Thinned May 2005 (age eight years) to 200 spha retaining the best-formed trees in the orchard

Key climatic parameters[7]

Parameter	**Expt 460a HWD**	**Expt 460b HWD**	**Expt 460e HWD**
Mean rainfall	682 mm	622 mm	682 mm
Median rainfall	668 mm	617 mm	668 mm
Mean maximum temperature hottest month	32.9°C	31.8°C	32.9°C
Mean minimum temperature coldest month	5.6°C	3.7°C	5.6°C
Mean number of rain days per year	67	68	67
Mean number of days frost (< 2.5°C)	19.2	38.3	19.2

[7] Key climatic parameters for the period 1889–2009 derived using SILO. (http://www.longpaddock.qld.gov.au/silo/datadrill/index.frames.html)

E. cloeziana

Site description

	Expt 481d HWD
Location	Cpt 56 St Marys LA SF 57 St Mary
Latitude/longitude	25.67°S 152.52°E
Elevation	60 m a.s.l.
Rainfall	Tiaro Post Office Mean 981 mm, median 1029 mm 11 years 1999–2009
Rainfall site	8.6 km south-east of trial site
Soil type	Red Earth/Red Podzolic
Date planted	May 2002
Spacing	5.0 m × 1.8 m = 1111 spha
Thinning history	Thinned to half stocking (555 spha) – April 2006

Key climatic parameters

Parameter	**Expt 481d HWD**
Mean rainfall	997 mm
Median rainfall	975 mm
Mean maximum temperature hottest month	31.2°C
Mean minimum temperature coldest month	7.7°
Mean number of rain days per year	92
Mean number of days frost (< 2.5°C)	5.4

Corymbia hybrids

Site description

	Expt 469d HWD	**Expt 394a HWD**	**Expt 394b HWD**
Location	Amamoor FPQ's Poulson's block Amamoor Ck Road	Devils Mountain, Sexton FPQ's Mulholland's block	Mt McEwan, Hivesville FPQ's Stumer's block
Latitude/longitude	26.36°S 152.54°E	26.05°S 152.46°E	26.21°S 151.70°E
Rainfall	Kandanga Post Office Mean 1182 mm Median 1126 mm 81 years 1919–2003	Theebine Mean 978 mm Median 993 mm 111 years 1894–2009	Proston Post Office Mean 713 mm Median 691 mm 71 years 1938–2009
Rainfall site	14.0 km east-south-east of trial site	14 km north-east of trial site	12.5 km north-west of trial site
Elevation	154 m a.s.l.	80 m a.s.l.	438 m a.s.l.
Soil type	Yellow Podzolic	Black Earth	Red Krasnozem
Date planted	14 September 2001	4 March 2003	27 February 2003
Spacing	4.0 m × 1.5 m = 1666 spha	4.0 m × 2.5 m = 1000 spha	4.0 m × 2.5 m = 1000 spha
Thinning history	unthinned	unthinned	unthinned

Key climatic parameters

Parameter	**Expt 469d HWD**	**Expt 394a HWD**	**Expt 394b HWD**
Mean rainfall	1109 mm	982 mm	707 mm
Median rainfall	1070 mm	942 mm	693 mm
Mean maximum temperature hottest month	30.4°C	29.8°C	30.4°C
Mean minimum temperature coldest month	6.4°C	6.2°C	4.3°C
Mean number of rain days per year	106	91	78
Mean number of days frost (< 2.5°C)	11.7	13.1	31.7

E. pellita

Site description

	Expt 767a ATH
Location	Forest Plantation Queensland's old nursery site 4.0 km south-west Ingham
Latitude/longitude	18.67°S 146.1425°E
Rainfall	Ingham Township Mean 2143 mm Median 2103 mm 39 years 1970–2010
Rainfall site	4.6 km north-east of trial site
Elevation	10 m a.s.l.
Soil type	Yellow Chromosol/Sodosol (? not described)
Date planted	29 May 1997
Spacing	5.0 m × 1.6 m = 1250 spha
Thinning history	Thinned to 625 spha at age two years and to 312 spha at age four years

Key climatic parameters

Parameter	**Expt 767a ATH**
Mean rainfall	2057 mm
Median rainfall	2054 mm
Mean maximum temperature hottest month	32.4°C
Mean minimum temperature coldest month	13.4°C
Mean number of rain days per year	113
Mean number of days frost (< 2.5°C)	0.0

Appendix 3a.

Inventory of grafted E. cloeziana clonal material selected from St Mary's progeny trial (481dHWD) for superior growth and desirable wood properties (a) and superior growth only (b).

a)

Clone	Ramets
1ec2-038	1
1ec2-042	1
1ec2-040	5
1ec2-031	32
2ec2-002	1
2ec2-003	3
1ec2-025	8
1ec2-030	3
1ec2-024	6

b)

Plot	Row	Tree	Entry	Provenanc	Ramets
70	2	3	192	Toolara	12
41	6	3	141	Mungy	2
28	2	3	9	Veteran	60
44	4	3	216	Veteran	57
51	1	5	183	Wolvi	67
36	6	5	133	Cannidah	0

Appendix 3b. Inventory of grafted E. argophloia clonal material selected from progeny trial (460cHWD) for superior growth only.

Row	Tree	Family	Provenance	Ramets
2	**12**	**79**	**Burncluth**	**5**
3	**14**	**34**	**Burncluth**	**1**
3	**34**	**31**	**Fairyland**	**3**
2	**38**	**62**	**Burncluth**	**1**
7	**22**	**37**	**Burncluth**	**2**
6	**22**	**76**	**Burra Burri**	**7**