Inter-specific *Corymbia* hybrid research; providing new opportunities for plantation expansion in northern Australia

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General Introduction

In northern Australia, much of the land available for hardwood plantation expansion is located in areas where environmental conditions (e.g. low/variable summer rainfall, frost, pest pressures and poor soils) are unfavourable to most recognised plantation species. The *Corymbia* group are an exception to this, with the spotted gums, (e.g. *Corymbia citriodora* subsp. *variegata* or CCV), currently providing some of the best options for these sites. Unfortunately spotted gum plantations are also often susceptible to disease and frost damage and are generally considered to be of only low-moderate productivity (Dickinson *et al*. 2004; Lee *et al*. 2006). However, recent advances in the development of interspecific *Corymbia* hybrids have produced families which have exceptional early performance across numerous sites in northern Australia.

Early results from the *Corymbia* hybrid research program have shown that some individuals and families significantly out-perform the best spotted gum provenance (CCV-Woondum), across a range of sites (Lee *et al.* 2005, Dickinson and Lee 2005; Lee *in press*). These inter-specific hybrids utilised *C. torelliana* (cadaghi) as the maternal parent and either *C. citriodora* subsp. *variegata* (spotted gum or CCV), *C. citriodora* subsp. *citriodora* (lemon-scented gum or CCC) or *C. henryi* (broad-leaved spotted gum) as pollen parents. Phenotypic characteristics contributing to this superior performance include; good tolerance to the disease *Quambalaria pitereka* (ramularia shoot blight), moderate – high insect tolerance to erinose mite (*Rhombacus* spp.), longicorn beetles (*Phoracantha* spp.) and red-shouldered leaf beetles (*Monolepta australis*), good frost tolerance and a high adaptability to a range of site types (Lee *in press*).

Currently the breeding systems of *Corymbia*, and in particular, compatibility between species are poorly understood, with varying degrees of success achieved between a limited range of section, species and family combinations. This paper describes two *Corymbia* hybrid research projects currently underway in Queensland. Part 1 identifies the most effective and efficient controlled pollination methodologies for this genus, to support future *Corymbia* hybridisation programs. Part 2 investigates the breeding systems of a wide range of crosses within the *Corymbia* genus, examines inter-specific compatibility/incompatibility mechanisms and creates new inter-specific hybrids with high plantation potential.

Part 1: Controlled-pollination methods

Many commercial plantation programs have used spontaneous hybrids, however better phenotypes will be produced through well structured, manipulated hybridization programs (Eldridge *et al.* 1993). Since the 1950's, controlled pollination methodologies have been developed for eucalypts, including the conventional pollination or CP method (Moncur 1995), the one-stop or OSP method (Harbard *et al*. 1999) and most recently the artificially induced protogyny (AIP) method (de Assis *et al*. 2005).

The CP method is the most intensive method, requiring three visits to the inflorescence whereby; (1) ripe buds are emasculated and exclusion bagged (2) bags are removed, pollen is applied to stigmas at the time of maximum receptivity and then re-bagged and (3) exclusion bags are removed after styles are no longer receptive (Moncur 1995). The OSP technique involves only two steps whereby; (1)

nearly ripe buds are emasculated, the style is decapitated to remove the stigma, pollen is applied to the cut surface and the flowers exclusion bagged and (2) exclusion bags are removed after styles are no longer receptive (Harbard *et al*. 1999). The AIP technique also involves two steps but is much quicker than the CP and OSP techniques as no emasculation is necessary. In this method (1) the tip of the operculum and stigma are removed from ripe buds with a single cut. Pollen is applied to the cut surface of the style and the flowers are exclusion bagged and (2) exclusion bags are removed after styles are no longer receptive (de Assis *et al*. 2005).

In this study, these three controlled pollination techniques were investigated, for their efficiency and effectiveness for use in a large-scale, commercial program for producing *Corymbia* hybrids. For each technique pollination speed and relative seed yields were assessed.

Methods

This experiment was conducted in 2005, with seven inter-specific controlled pollination treatments (treatments 1-7) and three intra-specific control treatments (treatments 8-10);

1. Controlled pollination (CP)
2. One-stop pollination (OSP)
3. One-stop pollination + bag (OSP + bag)
4. Artificially-induced protogyny – Ripe buds (AIP ripe)
5. Artificially-induced protogyny – Ripe buds (AIP ripe + bag)
6. Artificially-induced protogyny – Green buds (AIP green)
7. Artificially-induced protogyny – Green buds (AIP green + bag)
8. Open pollination (Open)
9. Self pollination using One-stop pollination + bag (Self)
10. Cross-pollination using One-stop pollination + bag (Cross)

Three *C. torelliana* trees (replicates) were used as the maternal parents, with a polymix of two CCV families used as the inter-specific pollen parent. A polymix of three unrelated *C. torelliana* families was used for the cross-pollinated control, with pollen sourced from the specific maternal parent used for the self-pollinated control.

On each *C. torelliana* tree, one bunch (60 – 85 flowers) per pollen treatment was conducted. Either semi-ripe green buds (AIP green buds) or ripe buds (all other treatments) were selected. The number of capsules/bunch was counted and the time taken to perform each operation was recorded. Specialised pliers were used to emasculate flowers in all the CP and OSP treatments, or to remove the tip of the operculum and style in the AIP treatments. For the OSP treatments, a scalpel blade was used to decapitate styles. Pollen was then applied using a match head to all treatments, except the CP treatment when pollen was applied seven days later in a separate visit. In specified treatments, pollinated flowers were covered with an exclusion bag for 14 days to prevent pollen contamination from other sources. Capsule retention rates were measured at harvest (10 – 13 weeks after pollination), seed was extracted and the average number of seed collected per capsule calculated for each treatment.

Results: Pollination speed

The time taken to perform all operations for each of the seven inter-specific pollination treatments was calculated on a per capsule basis (Figure 1). These results indicate little difference in pollination speed between the CP, OSP and OSP + bag treatments. All four AIP treatments however, were faster than the CP and OSP treatments, with the two AIP green bud treatments marginally quicker than the AIP ripe bud treatments. For all AIP treatments, there was little time difference between those with or without bagging.

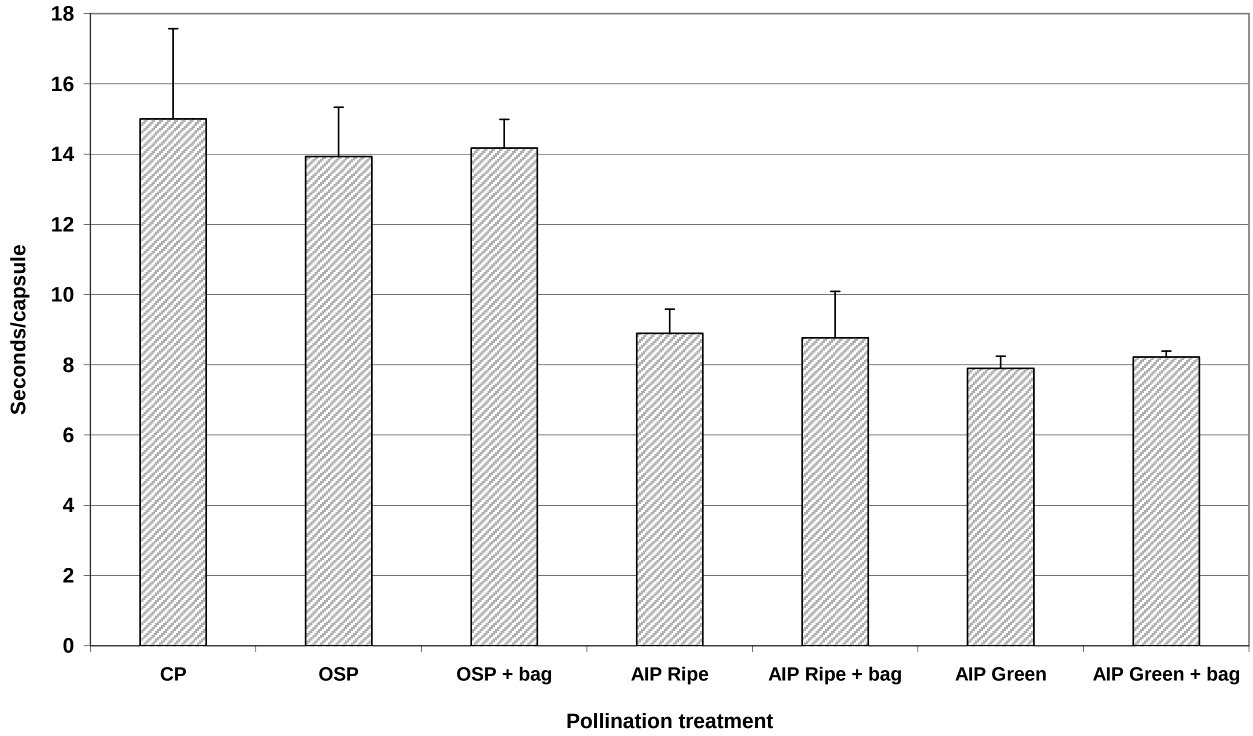

Figure 1: Pollination time (seconds/capsule) for each pollination treatment (CP = Controlled pollination, OSP = One-stop pollination, AIP = Artificially induced protogyny). Bars indicate standard errors.

Results: Seed yields

Seed yields per treatment (seed produced/100 pollinated capsules) were calculated from the data of number of capsules pollinated, number of capsules retained at harvest and average seed number per capsule (Figure 2). The highest seed yields were obtained in the two AIP ripe buds treatments and the open and cross pollinated treatments. Moderate seed yields were recorded for the OSP and OSP + bag treatments, although bag exclusion did result in lower seed yields. The CP and the two AIP green buds treatments had quite low seed yields, while the self-pollinated treatment had the lowest seed yields.

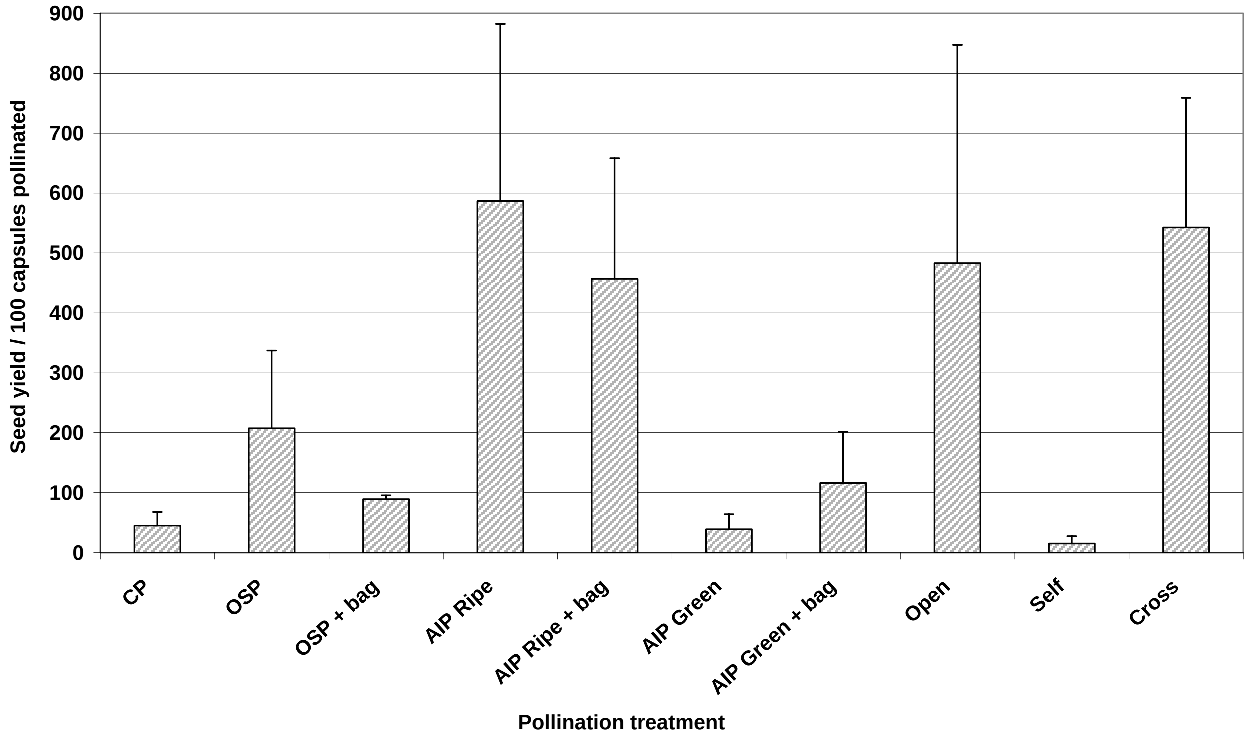

Figure 2: Seed yields (seed number/100 capsules pollinated), for each pollination treatment (CP = Controlled pollination, OSP = One-stop pollination, AIP = Artificially induced protogyny). Bars indicate standard errors.

Discussion

Pollination speed

Operator speed was much faster for the AIP treatments than either the OSP or CP treatments. This was mainly because the time-consuming emasculation operation is not undertaken during AIP. In addition, the delicate styles are less exposed in the AIP method, making the treated flowers more robust and easier to handle. Time differences between the CP and OSP treatments were not obvious, as while the CP treatment required an additional bagging operation, time was saved as it was not necessary to cut the styles as was conducted for the OSP treatment.

Time was only recorded when the operator was positioned at the bunch, hence in treatments where more than one visit was necessary, the additional time taken to revisit and set-up prior to operator handling was not taken into account. This applied to the three treatments with exclusion bagging (OSP + bag, AIP ripe + bag and AIP green + bag) which required two visits and the CP treatment which required three visits. In practice this would add additional time and cost to these treatments.

Seed Yields

Seed yields were amongst the highest from the two *C. torelliana* x *C. torelliana* control treatments (open-pollinated and cross pollinated), with approximately 500 seeds/100 capsules pollinated. The *C. torelliana* self-pollinated treatment however, produced only very low seed yields, indicating that the trees tested were not highly self compatible and that contamination through self pollination (which is a risk in the AIP technique) should be very minimal.

Within the seven inter-specific controlled pollination treatments, the two AIP ripe treatments produced high seed yields, similar to those measured for the best intra-specific controls. While there is a high potential of external *C. torelliana* pollen contamination within the un-bagged treatment, the majority of seed within the bagged treatment, should be the *C. torelliana* x CCV hybrid. For both the AIP green buds treatments, seed yields were low in comparison, indicating that green buds are unsuitable for this pollination method.

The OSP and CP treatments had lower seed yields than the AIP treatments, which suggests the emasculation process has some detrimental effects on capsule set and seed production. During and after emasculation, there is a risk of style and capsule damage, this may influence seed yield. Exclusion bags may also damage delicate styles, this may have contributed to lower seed yields in the bagged treatments, however it is expected that un-bagged treatments are at higher risk of *C. torelliana* pollen contamination.

The low seed yield with the CP treatment may be due to a variation in maturity across flowers within the bunch at the time of pollination, with not all stigmas highly receptive to pollen at the same time. In this study only one pollination visit was conducted however, Moncur (1995) indicates that pollination may need to be conducted over three separate visits to ensure optimum fertilisation rates.

Conclusions

The four AIP pollination treatments were quicker to conduct than either the CP or OSP methods. Much of the time saved is through omitting emasculation and faster handling as treated capsules are less delicate. The use of exclusion bags in the OSP and AIP methods, did not add significant time to either technique.

Both of the AIP ripe treatments were very successful, resulting in seed yields equivalent to the intra-specific open and cross pollinated control treatments. Contamination levels are yet to be determined; however as the *C. torelliana* trees showed low self compatibility, most seed produced should be from cross fertilisation. Hence in the AIP ripe + bag treatment, where external *C. torelliana* pollen contamination was restricted, most seed produced should be from the *C. torelliana* x CCV cross. The CP, OSP and OSP + bag treatments produced much lower seed yields in comparison.

The AIP ripe + bag treatment was quick and while pollen contamination levels still need to be determined, this method has produced the highest yields of *C. torelliana* x CCV hybrid seed.

Part 2: New inter-specific hybrid combinations

The *Corymbia* is a new genus split-off from the genus *Eucalyptus* (Hill and Johnson 1995), creating much taxonomic controversy (Brooker 2000; Ladiges and Udovicic 2000). The *Corymbia* comprises over 113 species within seven extracodical sections, and are mainly distributed in the tropical and sub-tropical regions of northern Australia. This genus contain recognised native timber species including *C. maculata, C. henryi, C. citriodora subsp. variegata, C. citriodora subsp. citriodora, C. torelliana, C. tessellaris* and *C. intermedia* (Boland 1984). Two species, *C. ptychocarpa* and *C. ficifolia* are also used in amenity horticulture. The genus *Corymbia*, particularly section *Politaria,* (the spotted gums) has been recognised with high potential for use within the expanding Australian plantation timber industry and a number of specific domestication programs have been initiated (Lee *in press*).

The *Corymbia* has a propensity to form natural inter and intra-sectional hybrids in nature (Griffin *et al.* 1988), with numerous inter-specific *Corymbia* hybrids (*C. torelliana* x CCV, CCC or CH) observed as spontaneous hybrids in amenity plantings in southern Queensland. These hybrids have been replicated in the current breeding program (Nikles *et al*. 2000; Lee *in press*). Using controlled pollination techniques we attempted to produce new inter-specific *Corymbia* hybrids using species previously untested as pollen parents. These additional species were tested as they were thought to provide additional desirable characteristics, including greater environmental adaptability, disease and insect resistance or timber quality than the suite of species already being tested as hybrid parents.

Methods

A series of three experiments were conducted; in 2005 (Experiments 1 and 2) and 2006 (Experiment 3), with controlled inter-specific hybrid crosses made using *C. torelliana* as the maternal parent and 16 species as pollen parents. Each experiment involved different inter-specific hybrid crosses, with outcrossed *C. torelliana* x *C. torelliana* as the control. The 16 pollen parents represented six of the seven *Corymbia* sections, as well as one species each from the related genera, *Eucalyptus* and *Angophora*. All pollen treatments involved a poly-mix of a minimum of three trees/species, except CCV and *C. henryi,* which included two trees/species (Table 1.) Pollen viability for each polymix was confirmed using the methods described by Moncur (1995).

Genus	**Section ***	**Series**	**Species**	**Expt. 1**	**Expt. 2**	**Expt. 3**
Corymbia	Apteria (1)	Trachyphloiae	*C. trachyphloia*	X		
"	Rufaria (67)	Intermediae	*C. intermedia*	X		
"	"	Ptychocarpae	*C. ptychocarpa*	X		
"	"	Dichromophloiae	*C. erythrophloia*	X		
"	"	Ficifoliae	*C. ficifolia*			X
"	"	Polycarpae	*C. clarksoniana*	X		
"	Ocharia (12)	Eximiae	*C. leichardtii*			X
"	Cadagaria (1)	Torellianae	*C. torelliana*	X	X	X
"	Politaria (4)	Maculatae	*C. citriodora* subsp. *citriodora*	X		
"	"	"	*C. citriodora* subsp. *variegata*		X	
"	"	"	*C. henryi*		X	
"	"	"	*C. maculata*			X
"	Blakearia (27)	Tessellares	*C. tessellaris*	X		
"	"	Grandifoliae	*C. dallachiana*	X		
Angophora	Liberia	Costatae	*A. floribunda*			X
Eucalyptus	Transversaria	Salignae	*E. pellita*			X

Table 1: Pollen treatments investigated in the three inter-specific hybrid experiments. (* number in brackets represents the total number of *Corymbia* species within each particular section)

For experiments 1, 2 and 3, a total of four, three and five *C. torelliana* trees respectively, were used as maternal parents. Each tree represented one replicate in the trial. On each tree, one bunch (40 – 100 flowers) per pollen treatment was pollinated using the one-stop pollination technique (Harbard *et al.* 1999). During pollination, flowers were emasculated using pliers, styles were decapitated using a scalpel blade and the pollen applied with a match head. Pollinated flowers were covered with an exclusion bag, for 14 days to prevent pollen contamination from other sources. Capsule retention rates were measured at harvest (10 – 13 weeks after pollination), seed was extracted and the average number of seed collected per capsule calculated for each treatment.

Results

For all three experiments, the number of seeds/capsule harvested, was highest in the outcrossed *C. torelliana* x *C. torelliana* control treatment (average 17 – 22 seeds/capsule). In order to provide a comparison between all species across the four separate experiments, the seed number/capsule measured for each treatment was calculated as a percentage of that measured for the *C. torelliana* x *C. torelliana* control treatment in that experiment. This data is presented in Figure 3 and provides a guide to the relative rankings of pollination success across the 16 pollen treatments.

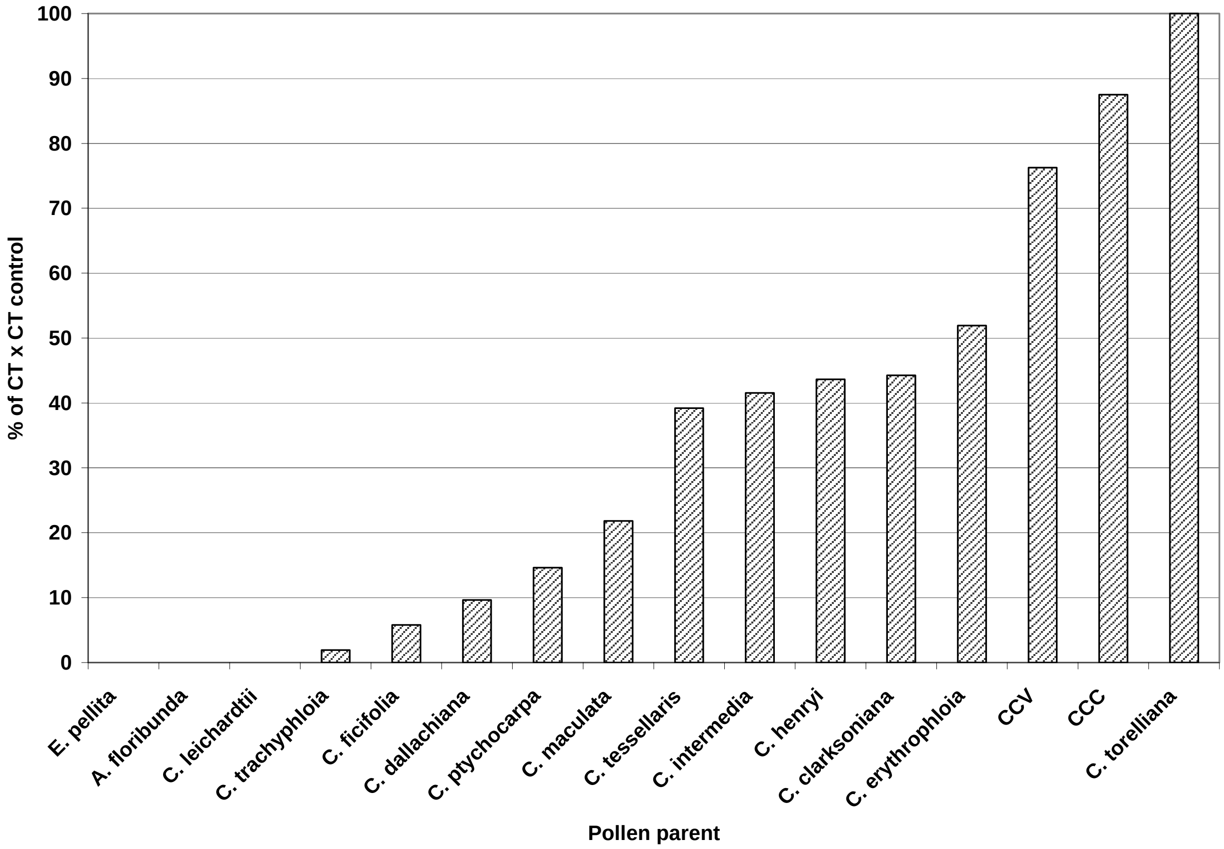

Figure 3: Relative seed yields/capsule for the 16 pollen parent species as compared to the *C. torelliana* x *C. torelliana* control.

Seed yields/capsule was highest within CCV and CCC, both from section *Politaria*. The two remaining species within *Politaria*; *C. henryi* and *C. maculata*, had moderate and low seed yields respectively. Within section *Rufaria*, *C. erythrophloia*, *C. clarksoniana* and *C. intermedia* had moderate seed yields/capsule, while *C. ptychocarpa* and *C. ficifolia* were low. Within section *Blakearia*, *C. tessellaris* had a moderate seed yield, whereas *C. dallachiana* was low. *C. trachyphloia* (section *Apteria*) had a very low seed yield and no seed at all was recorded for *C. leichardtii* (section *Ochraria*), *Eucalyptus pellita* and *Angophora floribunda*.

Discussion

The trends in relative seed yields and hence inter-specific hybrid compatibility appear reasonably well associated with taxonomic affinity. Based on the cladistic analysis of *Corymbia* taxonomic affinity by Hill and Johnson (1995), the relatedness of the sections examined in this study to section *Cadagaria* (closest to farthest); is *Politaria, Ochraria, Blakearia, Rufaria, Apteria, Angophora* and *Eucalyptus*.

In this study, the highest seed yields were recorded in crosses between the closely related sections *Cadagaria* and *Politaria*. While this inter-sectional hybrid has not been recorded in the wild (their natural distribution rarely overlaps), numerous spontaneous hybrids have been identified, where seed had been sourced from *C. torelliana* trees, planted in close proximity to *Politaria* trees such as CCV and CCC. Lower seed yields with *C. henryi* and *C. maculata* may reflect a lower specific hybridising compatibility.

C. leichardtii, the only species from section *Ochraria* included in this study, did not appear compatible with *C. torelliana*, with no seed produced. Natural inter-sectional hybrids have however been recorded between the closely related *Politaria* and *Ochraria*, so it may be possible that other *Ochraria* species, undertaking the cross in the other direction (using *C. torelliana* pollen on *C. leichardtii*) or using different *C. leichardtii* trees as pollen parents may result in greater success in the future.

Cadagaria and *Blakearia* have been documented as producing natural inter-sectional hybrids, specifically *C. torelliana* x *C. tessellaris* (Hill and Johnson 1995). In this study, this hybrid combination was also successful producing moderate seed yields. Seed yields of *C. dallachiana* however, were poor which may indicate specific hybridising incompatibility.

C. erythrophloia, *C. clarksoniana* and *C. intermedia* from within section *Rufaria* produced moderate seed yields, indicating a promising inter-sectional compatibility with section *Cadagaria*. No natural *Cadagaria* x *Rufaria* inter-sectional hybrids have been recorded; however *Rufaria* x *Politaria* (*C. intermedia* x *C. maculata*) has been documented (Hill and Johnson 1995). As the *Rufaria* comprises 67 species and is the largest of the seven *Corymbia* sections, it would be worthwhile to further investigation of other inter-sectional hybrid combinations from this group.

The uni-specific section *Apteria* (*C. trachyphloia*) produced low seed yields, which indicates a low compatibility with the *C. torelliana* trees tested. The two inter-generic crosses conducted in this study; *C. torelliana* x *E. pellita* and *C. torelliana* x *A. floribunda* did not yield any seed. This was expected as there are no reliable records that inter-generic hybrids have ever occurred with the eucalypt group (Griffin *et al*. 1988, Potts *et al.* 2003). These two combinations were included in this study due to the current taxonomic debate over the generic classification of the *Angophora, Corymbia* and *Eucalyptus* groups (Brooker 2000; Ladiges and Udovicic 2000) and anecdotal reports of manipulated hybrids of *C. torelliana* x *E. pellita* and *E. grandis* x *C. torelliana* from overseas (Griffin *et al*. 1988).

Conclusions

This study has provided information on the potential compatibility of a number of new inter-specific and inter-sectional *Corymbia* hybrid combinations. It is hoped that in future progeny trials, these new hybrid combinations, will offer a unique set of phenotypic characters suited to plantation use, that are not available from known species and hybrids. In particular, sections *Rufaria* (including *C. intermedia* and *C. clarksoniana*) and section *Blakearia* (including *C. tessellaris*) have many desirable characteristics to offer to a hybrid combination, including high drought tolerance and a wide adaptability to many of the soil and climatic types in eastern and northern Australia.

Where hybrid crosses have been unsuccessful, it may be due to incompatibility (e.g. inter-generic *Corymbia* x *Eucalyptus* and *Corymbia x Angophora* crosses*)* or it may be due to specific tree incompatibility, whereas other trees from the same species may produce greater success. As suggested by the literature, the *Corymbia* have a high hybridizing capability and it is likely that further investigation of these and other untested *Corymbia* species combinations may provide new inter-specific *Corymbia* hybrid progeny for future testing and evaluation.

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