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Controlled Pollination Methods for Creating *Corymbia* Hybrids

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Abstract

Inter-specific *Corymbia* hybrids are of increasing interest to plantation forestry, yet there is little knowledge of the most suitable controlled pollination methods for this genus. Inter-specific crosses were made between *C. torelliana* [CT(maternal parent)] and *C. citriodora* subsp. *variegata* (CCV), *C. henryi* (CH) and *C. citriodora* subsp. *citriodora* (CCC) using conventional pollination, one-stop pollination (OSP) and artificially-induced protogyny on yellow buds (AIP Y) pollination methods. Additional treatments included AIP on green buds (AIP G) and the use of exclusion bags for the OSP and AIP methods. Inter-specific hybrids (CT x CCV, CT x CH and CT x CCC) were successfully created using all three pollination methods. The AIP Y treatment provided the highest seed yields and achieved time savings of >41% over the conventional and OSP methods, resulting in up to five-fold increases in operator productivity. However, the AIP Y treatment also had the highest *C. torelliana* contamination levels (9.3–13.2%). The use of exclusion bags with the AIP method had minimal effect on contamination rates, indicating a high proportion of self-pollen contamination. Contamination rates varied between maternal parents, suggesting variation in self-compatibility for *C. torelliana* individuals. AIP using semi-ripe green buds was not effective at reducing selfing and had low operator productivity. The AIP method is suitable for use in a large-scale hybrid breeding program for *C. torelliana*. When self-pollination effects are managed, it could greatly reduce the costs associated with the production of seed of elite family crosses for commercial forestry deployment.

Key words: Conventional pollination, one-stop pollination, artificially-induced protogyny, self-pollination, spotted gum, *Eucalyptus*, inter-specific hybrid.

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Introduction

Inter-specific Corymbia (formerly *Eucalyptus* subgenus *Corymbia*) hybrids have recently shown great promise for future expansion of hardwood plantations in Australia and overseas (LEE 2007; TRUEMAN and RICHARDSON 2008; LEE *et al*., 2009). These hybrids originated from crosses using *C. torelliana* (CT) as the maternal parent and *Corymbia* species [primarily, *C. citriodora* subsp. *citriodora* (CCC), *C. citriodora* subsp. variegata (CCV) or *C. henryi* (CH)] from within section *Politaria* (spotted gums) as the pollen parent. Hybrids of exceptional performance were first identified as rare, spontaneous hybrids within *C. torelliana* windbreaks and amenity plantings in Australia (NIKLES *et al*., 2000). Progeny trials established in north-eastern Australia have since demonstrated that *Corymbia* hybrids have many advantages over their spotted gum paternal parents, which are highly susceptible to the disease *Quambalaria pitereka* (DICKINSON *et al.*, 2004; JOHNSON *et al*., 2009). These advantages include superior growth, disease, insect, and frost tolerance, exhibited across a wide range of environments (LEE, 2007; LEE *et al*., 2009).

Hybridization programs for eucalypts often use a conventional pollination technique to produce intra- and inter-specific hybrid seeds (ELDRIDGE *et al.*, 1993; DELAPORTE *et al*., 2001). Conventional pollination relies on the protandrous nature of eucalypts, and involves three visits to the inflorescence whereby: (1) immature buds and open flowers are removed and the ripe buds are emasculated and exclusion bagged, (2) pollen is applied to receptive stigmas and the flowers are re-bagged, and (3) exclusion bags are removed after the styles have withered (VAN WYK, 1977; MONCUR, 1995). This method has been used to produce inter-specific *Corymbia* hybrids for the floriculture industry (HENRY, 1995; SEDGLEY and DELAPORTE, 2004). Conventional pollination however, is often considered too time consuming and expensive for larger-scale commercial hybridisation programs (VAN WYK, 1977; MONCUR, 1995).

The one-stop pollination (OSP) technique increases operator productivity while producing comparable seed yields to the conventional pollination method (HARBARD *et al.*, 1999; WILLIAMS *et al*., 1999). In the OSP technique, the immature buds and open flowers are removed, mature buds are emasculated, and the style is cut to remove the stigma. Pollen is then immediately applied to the cut surface. The style is then isolated to prevent contamination with foreign pollen, using either tubing on individual flowers or exclusion bags for multiple flowers. With less return visits to the inflorescence, this method is cheaper than conventional pollination and has increased both the research and commercial development and deployment of hybrid seed (POTTS and DUNGEY, 2004).

Artificially induced protogyny (AIP) is a recently developed method of controlled pollination (ASSIS *et al*., 2005), which has produced greater productivity rates than the OSP method for some *Eucalyptus* species (HORSLEY *et al*., 2010). This technique does not require emasculation, involving a cut to remove the tip of the operculum and the stigma, with pollen then immediately applied to the cut surface of the upper style. Exclusion bags are then used to prevent contamination with foreign pollen until the style is not receptive. However, as emasculation is not performed, there is an increased likelihood of self pollination occurring, particularly where highly self-compatible species are used (ASSIS *et al*., 2005). Strategies that have been assessed to minimise the levels of pollen contamination when using the AIP method include the use of semi-ripe, green buds (ASSIS *et al*., 2005; HORSLEY *et al*., 2010) and post-pollination application of sodium alginate gel to the cut style surface (HORSLEY *et al*., 2009).

In this study we aimed to identify the most suitable controlled pollination technique for creating inter-specific *Corymbia* hybrids, by comparing the effects of conventional, OSP and AIP controlled pollination methods on seed yield, operator productivity and genetic contamination.

Materials and Methods

Experimental design

Two randomised complete block design experiments were conducted to investigate the effectiveness and efficiency of controlled pollination methods for creating inter-specific *Corymbia* hybrids, by crossing CT with CCV and CH in experiment 1 and crossing CT with CCC in experiment 2. All maternal parent trees (of unknown genetic origin) were selected from amenity plantings near Mareeba (17°00'S, 145°43'E), Queensland, Australia. Trees were greater than 8 m tall and within 50 m of other *C. torelliana* trees. Three replicates (maternal parent trees) were used for experiment 1 in 2005 and four replicates (maternal parent trees) were used for experiment 2 in 2006. Within each replicate, each pollination treatment was applied to a single flower bunch (50–70 buds), to obtain a mean treatment result for that replicate.

Pollen was collected from two CCV, two CH, and four CCC paternal parents. Flowers were collected prior to opening and placed into vases containing water, and anthers were harvested after the operculum was shed. The pollen was then extracted, dried for 72 hours in a silica-gel desiccator at room temperature, placed into gel capsules and stored with silica gel at 4°C. A pollen polymix was made for each parent species, with pollen viability confirmed two weeks prior to pollination, using methods described by MONCUR (1995).

Five pollination treatments were investigated in both experiments: 1) conventional pollination; 2) OSP; 3) OSP + bag; 4) AIP Y (yellow buds); and 5) AIP Y + bag. In experiment 1, two additional treatments, 6) AIP G (green buds) and 7) AIP G + bag, were also included (*Table 1*). All pollinations were carried out between August and October, with flowers accessed using an 8 m tall elevated platform. Each pollination treatment was conducted on a single flower bunch on each maternal parent (replicate), using either semi-ripe buds (AIP G and AIP G + bag treatments) or ripe buds (all other treatments). The semi-ripe bunches contained a majority of buds that were light green and within 3–7 days of natural operculum lift. The ripe bunches contained

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Table 1. – Controlled pollination treatments and total number of buds pollinated for *Corymbia torelliana* (CT) x *C. citriodora* subsp. *variegata* (CCV), CT x *C. henryi* (CH) and CT x *C. citriodora* subsp. *citriodora* (CCC) hybrid combinations in experiments 1 and 2.

Treatment details		No. of buds pollinated per pollen parent		
		Experiment 1		Experiment 2
Abbreviation	Pollination method description	CCV	CH	CCC
1) Conventional	Conventional pollination	209	233	217
2) OSP	One-stop pollination	201	222	230
3) OSP + bag	One-stop pollination + bag	239	209	213
4) AIP Y	Artificially-induced protogyny – yellow buds	206	224	199
5) AIP Y + bag	Artificially-induced protogyny – yellow buds + bag	226	217	229
6) AIP G	Artificially-induced protogyny – green buds	230	231	–
7) AIP G + bag	Artificially-induced protogyny – green buds + bag	233	243	–

mostly yellow buds within 0–3 days of natural operculum lift. Once a bunch was selected, open flowers and immature, overripe and excessive buds were removed, retaining approximately 50–70 buds per bunch.

Flowers were emasculated with specialised forceps for the conventional and OSP treatments. For the OSP treatments, stigmas were removed using a scalpel blade and pollen applied to the cut surface of the style using a match stick. For the conventional pollination treatment, the emasculated flowers were covered with a polyester pollination bag. Approximately seven days later, the stigmas had visible exudates and were then pollinated. Within the AIP treatments, the tip of the operculum was cut using a scalpel blade, to a depth which also removed the stigma. Pollen was then applied to the cut surface of the style. For all treatments, the number of buds pollinated per bunch was recorded and each bunch labelled with an identity tag. For treatments which included bag exclusion, a polyester bag was placed over the pollinated buds and removed after 14 days.

Data collection

The time required for the operator to perform each pollination component (deflower-style preparation, cut style, pollinate, count-tag-bag and final de-bag) within each treatment, was recorded using a stop-watch, once the operator was positioned and could handle the flowers directly. In experiment 1, these results for the CT x CCV and CT x CH crosses were combined. Operator speed per bud was then calculated for each pollination treatment.

Capsule retention rates for each bunch were measured 2 weeks after pollination and again at maturity 11–14 weeks after pollination. Mature capsules were harvested and air-dried for a minimum of 7 days, prior to seed extraction. Operator productivity rates (seeds produced per hour of pollinating) were calculated for each treatment using the data for operator speed per bud and seed yield per bud pollinated.

C. torelliana pollen contamination was calculated for all treatments in experiment 1, except the AIP G and AIP G + bag treatments for the CT x CH hybrid combination, which had no viable germinants. For both the CT x CCV and CT x CH hybrid combinations, a maximum of 100 seeds from each pollination treatment and each maternal parent were randomly chosen, sown onto germination trays and placed into a germination cabinet where they were incubated at 25 °C. A random subset (maximum 20 trees) from each sample was then planted into line plots that were arranged in a randomised complete block design in the field near Mareeba. When the seedlings were 15 months old (11 months after planting), individuals were identified as either inter-specific *Corymbia* hybrids or as *C. torelliana* based on morphological differences.

Statistical analysis

All data was screened for assumptions of normality and homogeneity of variance, prior to analysis. Where necessary, proportion data was arcsine-transformed to convert to a normal distribution and numerical data was log-transformed to correct for unequal variances. Statistical analysis was conducted using Genstat 8.1 statistical software (GENSTAT, 2005), using a general analysis of variance (ANOVA) with randomised blocks. Where F values were significantly different ($P < 0.05$), comparisons between means were conducted using the 'protected' Least Significant Difference (LSD) test.

Results and Discussion

Operator speed

In both experiments, the AIP method provided significantly faster operator speeds per bud ($P < 0.001$) than all other treatments, with time savings of up to 41% and 47% respectively over the OSP and conventional pollination methods (*Table 2*). Within AIP treatments, bud maturity at pollination (semi-ripe, green buds or ripe, yellow buds) and the use of exclusion bags had little effect on operator speed. ASSIS *et al*. (2005) also reported increased operator speed as a major benefit of the AIP method, with, time savings of 70% and 90% respectively over the OSP and conventional pollination methods using smaller flowered *E. urophylla*, *E. grandis* and *E. grandis* x *E. urophylla* hybrids as maternal parents. As smaller flowers are more difficult to handle, the time benefits of AIP will increase as flower size decreases.

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Table 2. – Operator speed (seconds/bunch) for each pollination component and operator speed (seconds/bud), within each treatment for experiments 1 and 2. Treatment means with different letters are significantly different ($P < 0.05$). OSP = one stop pollination, AIP = artificially induced protogyny, Y = yellow buds, G = green buds.

Technique	Buds / bunch	Seconds / bunch						Seconds / bud
		Deflower & style preparation	Cut style	Pollinate	Count-tag-bag	Final de-bag	Total	
Experiment 1								
Conventional	73.7	617 a*	–	286 a^	98 a	19.8	1020 a	14.3 a
OSP	70.5	417 b	221	225 bc	37 b	–	900 b	12.9 a
OSP + bag	74.7	429 b	247	217 c	84 a	19.3	997 a	13.5 a
AIP Y	71.7	282 c	–	261 abc	41 b	–	584 c	8.2 b
AIP Y + bag	73.8	296 c	–	228 bc	94 a	19.7	638 c	8.7 b
AIP G	76.8	260 c	–	270 ab	48 b	–	578 c	7.6 b
AIP G + bag	79.5	253 c	–	274 a	66 ab	20.0	614 c	7.8 b
P value	0.780	< 0.001	–	0.017	< 0.001	–	< 0.001	< 0.001
LSD ($P < 0.05$)	na	76.3	–	45.1	32.8	–	96.0	1.8
Experiment 2								
Conventional	54.2	465 a*	–	260 ^	126 a	22.3	874 a	16.1 a
OSP	57.5	413 ab	149	219	49 bc	–	830 a	14.5 a
OSP + bag	53.2	414 ab	160	191	83 b	19.8	866 a	16.3 a
AIP Y	49.8	294 c	–	222	40 c	–	556 b	11.2 b
AIP Y + bag	57.2	357 bc	–	218	83 b	19.5	677 b	12.0 b
P value	0.541	0.01	–	0.096	0.004	–	< 0.001	< 0.001
LSD ($P < 0.05$)	na	86.4	–	na	39.3	–	126	1.9

*Includes bagging & first count, ^Includes debagging.

Compared with other pollination methods, the AIP method required significantly less time to conduct the de-flower – style preparation component ($P < 0.01$), as removal of the operculum tip and enclosed stigma is much simpler and faster than flower emasculation (*Table 2*). Substantial time was also saved because style preparation and cutting is performed as one operation. With the AIP method, the delicate styles remain largely protected by the surrounding inflexed stamens and the remaining operculum during the pollination process. This makes the treated buds more robust and easier to handle, particularly during pollination and bagging, resulting in cumulative time savings over the conventional pollination and OSP methods (*Table 2*).

Most pollination components were significantly slower ($P < 0.05$) using the conventional pollination method (*Table 2*). This was due to the extra time involved in bagging and greater operator difficulty in handling the delicate, naturally ripened and extended styles. An exception was the cut style component, which is unnecessary in conventional pollination, and added time to the OSP method. As a result, overall operator speed (seconds/bud) for the conventional pollination treatment was not significantly different ($P < 0.001$) from the OSP and OSP + bag treatments in both experiments (*Table 2*).

In this study, where pollination treatments included exclusion bagging, the additional time needed to revisit and set up the elevated platform to access the flowers could not be accurately measured and was not included in this analysis. In practice, the use of exclusion bags increases the time taken and costs for these treatments. In a large study when time logging over a commercial season was measured (HARBARD *et al*., 1999), the OSP method (without bag exclusion) achieved a 50% time saving compared with conventional pollination. In a pot-trial, ASSIS *et al*. (2005) reported a 65% time savings for the OSP method (without bag exclusion) compared to conventional pollination.

Capsule retention

Pollination treatments had a significant effect on mean capsule retention at 2 weeks post pollination. The lowest mean capsule retention was the AIP G treatment ($P < 0.05$) within the CT x CH crosses and the conventional pollination treatment ($P < 0.01$) for the CT x CCC crosses (*Table 3*).

At maturity, mean capsule retention rates were highest ($P < 0.05$) in the AIP Y and AIP Y + bag treatments within the CT x CCV crosses and the AIP Y and OSP treatments within the CT x CH crosses (*Table 3*). The higher capsule retention rates for the AIP Y method may be attributed to reduced physical damage to the delicate flower parts during pollination, due to elimination of the emasculation process and the temporary protection provided by the surrounding inflexed stamens and remaining operculum. Greater exposure to self and cross pollen contamination (particularly in unbagged treatments) may also explain higher capsule retention rates for these treatments. Equivalent or higher capsule retention rates using the AIP Y method (as compared to conventional pollination and OSP methods) have been reported for *E. grandis* (HORSLEY *et al*., 2010) and were attributed to increased pollen contamination and reduced physical damage.

The use of green semi-ripe buds (AIP G and AIP G + bag treatments) resulted in significantly lower

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Table 3. – Mean capsule retention (%) and mean seed yields for pollination treatments within each *Corymbia torelliana* (CT) x *C. citriodora* subsp. *variegata* (CCV), CT x *C. henryi* (CH), and CT x *C. citriodora* subsp. *citriodora* (CCC) hybrid combination. Treatment means with different letters were significantly different ($P < 0.05$). OSP = one stop pollination, AIP = artificially induced protogyny, Y = yellow buds, G = green buds.

Treatment details	Capsule retention %		Seed yield	
	2 weeks	Maturity	Seeds / capsule	Seed / bud pollinated
CT × CCV				
Conventional	51.7	5.6 c	11.3	0.5 c
OSP	78.9	19.6 abc	11.6	2.1 bc
OSP + bag	60.0	8.1 bc	13.8	0.9 c
AIP Y	58.2	34.4 ab	20.2	5.9 a
AIP Y + bag	85.9	39.0 a	12.6	4.6 ab
AIP G	27.5	6.2 c	10.5	0.4 c
AIP G + bag	57.0	15.2 bc	13.7	1.2 bc
P value	0.137	0.035	0.275	0.029
LSD ($P < 0.05$)	na	T^	na	3.5
CT × CH				
Conventional	55.4 a	9.4 bc	19.5 a	2.0 b
OSP	84.4 a	29.0 ab	6.7 bc	2.0 b
OSP + bag	54.9 a	15.9 bc	8.0 b	1.4 b
AIP Y	59.1 a	50.7 a	9.7 b	4.9 a
AIP Y + bag	65.7 a	19.0 bc	6.7 bc	1.4 b
AIP G	7.7 b	0.0 c	–	0.0 b
AIP G + bag	52.6 a	2.8 c	2.8 c	0.1 b
P value	0.020	0.005	0.004	0.015
LSD ($P < 0.05$)	35.9	22.10	5.0	2.4
CT × CCC				
Conventional	39.4 b	15.5	10.0	1.6
OSP	84.4 a	18.5	12.4	2.3
OSP + bag	73.6 a	25.0	11.9	2.9
AIP Y	84.1 a	27.9	10.2	3.0
AIP Y + bag	89.5 a	29.3	8.4	2.5
P value	0.007	0.379	0.748	0.856
LSD ($P < 0.05$)	25.4	na	na	na

(T^ = analysed using transformed data).

($P < 0.05$) capsule retention rates within both the CT x CCV and CT x CH crosses (*Table 3*). The results are similar to *E. grandis* and various *E. grandis* hybrids (Assis *et al*., 2000; Horsley *et al*., 2010). Capsule retention rates were also low for the conventional pollination and OSP + bag treatments within both the CT x CCV and CT x CH crosses. Lower capsule retention rates may be due to increased physical damage through increased operator handling (including placement and removal of exclusion bags). The conventional pollination treatment is also a more complex method, as it relies on the synchronous timing of pollination with natural stigma receptivity (Tibbits, 1989) and some stigmas may not have been at peak receptivity at pollination, which could contribute to lower capsule retention rates.

Seed yields

In this study, significant differences ($P < 0.05$) in mean seed number per capsule were found within the CT x CH crosses only, where the conventional pollination treatment was the highest and the AIP G + bag treatment the lowest (*Table 3*). Higher seed number per capsule for the conventional pollination method as compared with the OSP method has also been reported for *E. grandis* (Assis *et al*., 2005). In our study, the higher seed number per capsule for the conventional pollination treatment may be due to pollen and stigma – style interactions, with CH pollen tube germination and growth more successful within undamaged CT stigmas and styles.

Seed yield per bud pollinated were mostly influenced by capsule retention rates, with significant differences ($P < 0.05$) between pollination treatments measured within the CT x CCV and CT x CH crosses (*Table 3*). Within the CT x CCV crosses, mean seed yield per bud pollinated was highest in the AIP Y and AIP Y + bag treatments and lowest in the AIP G, AIP G + bag, conventional pollination, and OSP + bag treatments. Within the CT x CH crosses, the AIP Y treatment mean was significantly higher than all other treatment means. There was no difference in seed yield per bud pollinated between the conventional pollination, OSP and OSP + bag treatments.

The use of semi-ripe, green buds for the AIP method was unsuccessful, with low capsule retention rates, low seed number per capsule and very low seed yield per

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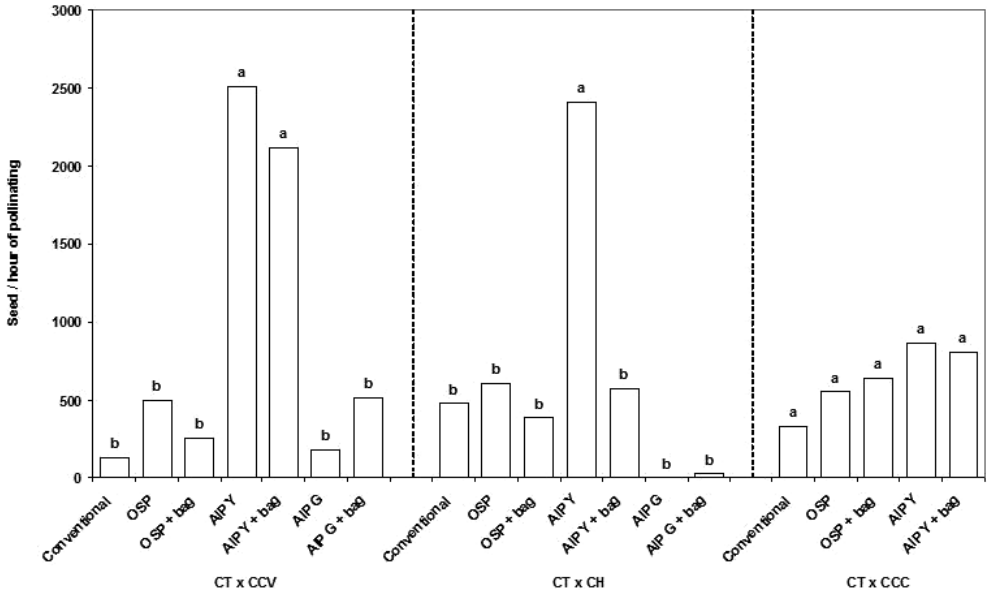

Figure 1. – Operator productivity (seed/hour of pollinating) for pollination treatments within each *Corymbia torelliana* (CT) x *C. citriodora* subsp. *variegata* (CCV), CT x *C. henryi* (CH), and CT x *C. citriodora* subsp. *citriodora* (CCC) hybrid combination. Treatment means with different letters are significantly different ($P < 0.05$). OSP = one stop pollination, AIP = artificially induced protogyny, Y = yellow buds, G = green buds.

bud pollinated. Similarly low yields for the AIP G method within *Eucalyptus* have also been reported (Assis *et al*., 2005 and Horsley *et al*., 2010). The use of green buds for the AIP method for *C. torelliana* is currently not viable.

Operator productivity

The AIP Y treatments had the highest operator productivity for all hybrid combinations (*Figure 1*). Within the CT x CCV crosses, the AIP Y and AIP Y + bag treatments were significantly more productive ($P < 0.05$) than other pollination treatments. Within the CT x CH crosses, differences were also significant ($P < 0.05$), with operator productivity highest in the AIP Y treatment and lowest in the AIP G and AIP G + bag treatments. In this study, faster operator speed and higher seed yields of the AIP yellow buds method resulted in up to a 5-fold increase in operator productivity over the conventional and OSP methods. Assis *et al*. (2005) also found substantially greater operator productivity between the AIP and OSP methods with a 3-fold increase recorded for *E. grandis*.

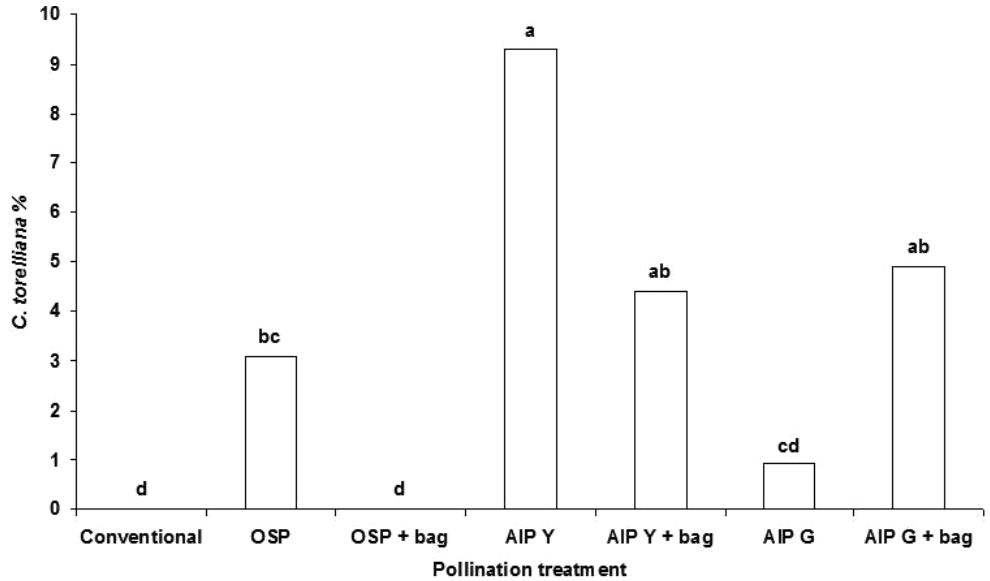

Figure 2. – *Corymbia torelliana* contamination for *C. torelliana* (CT) x *C. citriodora* subsp. *variegata* (CCV) pollination treatments in experiment 1. Treatment means with different letters are significantly different ($P < 0.05$). (OSP = one stop pollination, AIP = artificially induced protogyny, Y = yellow buds, G = green buds).

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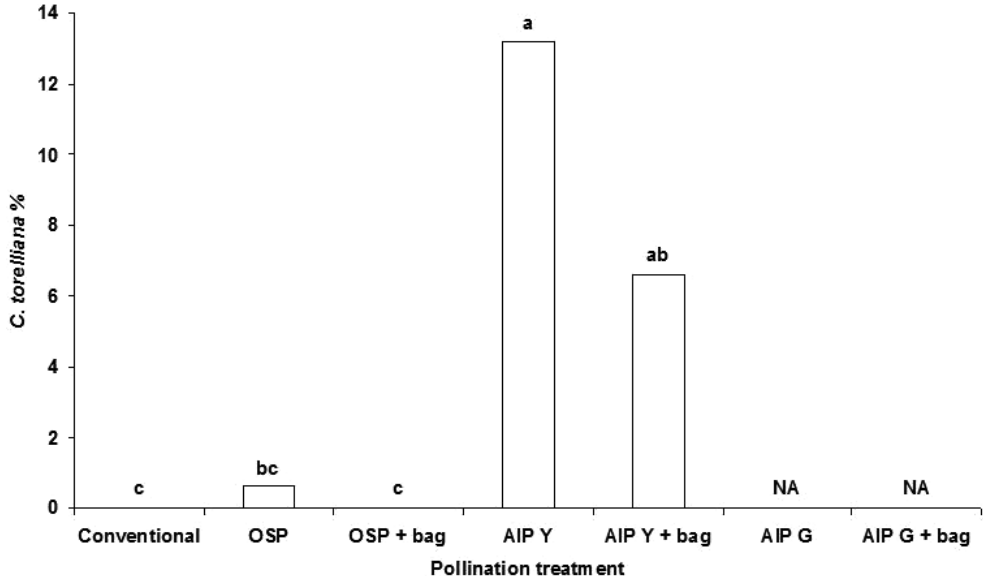

Figure 3. – Corymbia torelliana contamination for *C. torelliana* (CT) x *C. henryi* (CH) pollination treatments in experiment 1. Treatment means with different letters are significantly different ($P < 0.05$). OSP = one stop pollination, AIP = artificially induced protogyny, Y = yellow buds, G = green buds.

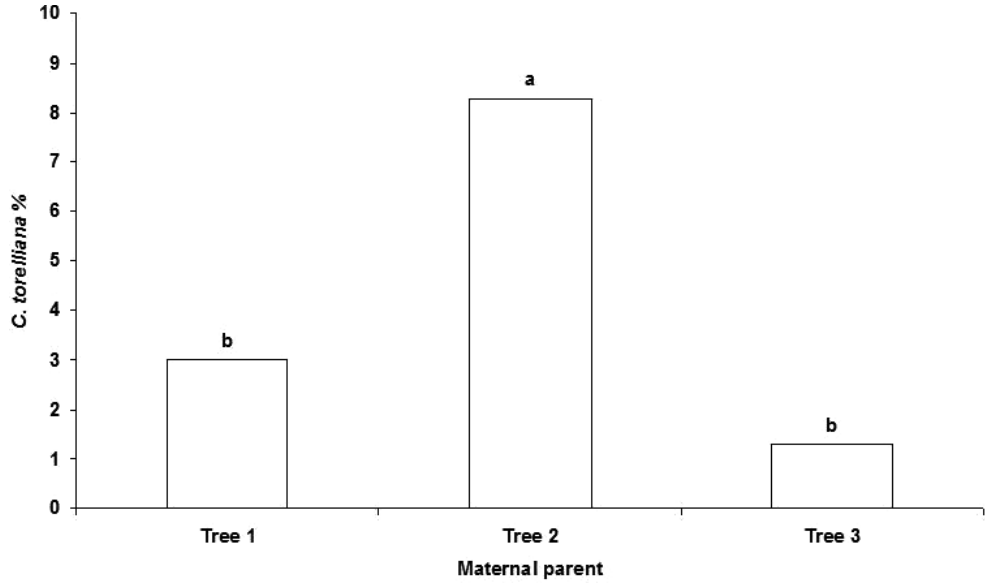

Figure 4. – Corymbia torelliana contamination for the three *C. torelliana* maternal parents used in experiment 1. Treatment means with different letters are significantly different ($P < 0.05$).

C. torelliana contamination

When seedlings were 15 months old, *C. torelliana* contamination was identified for all controlled pollination treatments from experiment 1, except the conventional and OSP + bag treatments (*Figures 2 and 3*). Within both the CT x CCV and CT x CH crosses, the AIP Y treatment had the highest contamination rate. This was significantly higher than all treatments ($P < 0.05$), except AIP Y + bag and AIP G + bag within the CT x CCV crosses and AIP Y + bag within the CT x CH crosses. The OSP treatment had intermediate *C. torelliana* contamination, with a rate significantly greater ($P < 0.05$) than the conventional and OSP + bag treatments within the CT x CCV crosses.

Within the unbagged AIP treatments, the high *C. torelliana* pollen contamination rates could be attributed to either self and cross sources. However where AIP with bag exclusion was used, *C. torelliana* contamination levels were still high, indicating that *C. torelliana* self pollen contributed greatly to the contamination rates. Increased likelihood of self pollination is one of the biggest disadvantages of the AIP method, particularly where highly self-compatible mothers are used (Assis *et al.*, 2005). The breeding system of most euca-

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lypt species is preferentially out-crossing (ELDRIDGE *et. al*., 1993; GRIFFIN *et. al.* 1987), with self-incompatibility mechanisms operating at both the pre- and post-zygotic stages (SEDGLEY and GRIFFIN, 1989). The AIP method may circumvent some of the pre-zygotic structural barriers, with HORSLEY *et al*. (2010) finding increased levels of self-compatibility within *E. grandis* controlled pollinations where AIP was used.

Differences in rates of *C. torelliana* contamination were also measured between the *C. torelliana* maternal parents, with tree 2 having significantly higher ($P < 0.05$) mean contamination rates than the other maternal parents (*Figure 4*). This tree may be genetically predisposed to higher self compatibility. Variation in self-compatibility rates between individuals of the same eucalypt species is well recognised (GRIFFIN *et. al*. 1987; ELLIS and SEDGLEY, 1992; POUND *et. al*., 2002). Highly-self compatible mothers are often eliminated from a breeding program to minimise the production of selfed seed (PATTERSON *et al*. 2004; POUND *et. al*., 2002).

HARBARD *et al*. (2000) suggested that a self-contamination rate of 10% is an acceptable level for commercial eucalypt seed production. The results from this study indicate that some *C. torelliana* individuals will exceed this rate when pollinated using the AIP method. Screening for self-compatibility could be used to exclude these highly self-compatible individuals from an AIP hybrid pollination program. If individuals of high self-compatibility are included in the breeding program, conventional or OSP pollination methods may be the most suitable techniques.

In this study, bagging excluded external *C. torelliana* pollen, with zero contamination rates measured for the conventional and OSP + bag treatments. In OSP treatments without exclusion bagging, contamination levels were still low (<4%). This is despite available out-cross and self pollen nearby, and pollen vectors, including *Trigona* spp., foraging on or near experimental styles. *Trigon*a spp. have a unique mutualistic relationship with *C. torelliana* and are highly attracted to resin from this species (WALLACE and TRUEMAN 1995, WALLACE *et al*., 2008). This low level of *C. torelliana* contamination may be due to three reasons: inter-specific pollen applied during pollination out-competed any foreign pollen; the cut style surface was only receptive to foreign pollen for a short time; or emasculated flowers were not attractive to pollinators. HARBARD *et al*. (2000), also reported low contamination levels (<12%) when OSP was conducted without exclusion bagging on *E. globulus*, despite styles remaining receptive for up to 4 days after style cutting and pollination.

Conclusion

Highest seed yield and greatest operator speed was achieved using the AIP Y pollination method on yellow mature buds (with or without exclusion bags), resulting in up to a five-fold increase in operator productivity, when compared to conventional and OSP methods.

Pollen contamination was also highest in the AIP Y treatments (with or without exclusion bags). Reducing contamination levels for the AIP method may be possible by excluding highly self-compatible *C. torelliana* mothers from AIP-mediated controlled pollination programs. If highly self-compatible individuals remain part of the breeding program, conventional or OSP pollination methods may be the most suitable pollination method. The use of semi-ripe green buds to minimise selfing during AIP was not an effective technique, resulting in low operator productivity rates with little effect on *C. torelliana* contamination levels. Exclusion bagging effectively prevented *C. torelliana* contamination in the conventional and OSP methods; however, contamination levels in the unbagged OSP treatments were low.

The AIP controlled pollination method has the potential to greatly reduce the costs associated with creation of elite Corymbia hybrid families, provided the risks of self-pollen contamination are addressed.

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