

Theme 111 "Family varieties"

Session 3: Mass production of improved seeds

Barriers to the production of interspecific hybrids in *Eucalyptus*

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Abstract

A detailed knowledge of crossability patterns is essential for the effective use of a hybrid breeding strategy as reproductive barriers may preclude many otherwise desirable species combinations from direct commercial exploitation. The major eucalypt subgenera are reproductively isolated and, despite natural hybridisation being relatively common, there is evidence for post-mating barriers of varying strength within subgenera. Many hybrid combinations result in high levels of deleterious abnormalities which may be expressed at various stages of the life cycle. Even in the case of hybridisation amongst the closely related species *Eucalyptus globulus*, *E. nitens* and *E. bicostata*, the level of deleterious abnormalities was significantly greater than outcross and inbred controls. Abnormalities in hybrid families were clearly evident in the nursery whereas the deleterious effects of inbreeding only became significant after 1 years' plantation growth. An optimum level of divergence associated with inter-provenance crossing was apparent for cross success and early growth. The expression of deleterious abnormalities in specific hybrid families was not predictable on the basis of the intraspecific performance of the parents and appears to be due to a different genetic mechanism to that resulting in inbreeding depression. The implications of such findings for hybrid breeding are addressed. In the case of the temperate species examined, the successful use of hybrids will depend on the development of efficient methods of clonal propagation as the operational production of F1 hybrid seed appears prohibitive.

Résumé

Il est essentiel de bien connaître les modèles d'appariement pour pouvoir utiliser efficacement la stratégie d'hybridation car des barrières à la reproduction sexuée peuvent limiter certaines combinaisons d'espèces intéressantes pour une production commerciale. Les principaux sous-genres d'*Eucalyptus* sont protégés bien que l'hybridation naturelle soit assez commune. Il existe évidemment des barrières plus ou moins fortes à l'intérieur des sous-genres. Plusieurs croisements hybrides présentent un degré élevé d'anomalie qui peut s'exprimer à différents stades de leur cycle. Même dans le cas d'hybridation d'espèces proches telles que *Eucalyptus globulus*, *E. nitens* et *E. bicostata*, le niveau d'anomalie est significativement plus élevé que les témoins issus de croisements intraspécifique ou d'autofécondation. En pépinière, les anomalies dans les familles hybrides

étaient nettes, alors que les effets non conformes dus à l'autofécondation ne sont apparus qu'après un an de plantation. Un niveau optimal de divergence pour la réussite de croisement est lié aux croisements entre provenances. Dans ces familles d'hybrides spécifiques l'apparition d'anomalies n'est pas prévisible à partir de la performance intraspécifique des parents et semble probablement due à des mécanismes différents de ceux provenant d'une dépression génétique consanguine. L'implication de tels résultats pour un programme d'amélioration basé sur l'utilisation d'hybrides est discutée. Chez des espèces tempérées, l'utilisation des hybrides est sous dépendance de méthodes efficaces de multiplication clonale, car la phase opérationnelle de la production de graines F1 apparaît prohibitive.

Keywords: *Eucalyptus*, *E. globulus*, *E. nitens*, *E. gunnii*, hybridisation, breeding barriers, crossing, inbreeding

1 Introduction

There is currently considerable interest in the use of interspecific hybridisation as a breeding strategy in *Eucalyptus* (Cauvin *et al.* 1987; Martin 1987, 1989; Kapoor and Sharma 1984; Pegg *et al.* 1989; Volker and Raymond 1989; Orme and Hetherington 1991; Vigneron 1991; de Little *et al.* 1992), yet little of the genetic or biological information to effectively assess or use a hybrid breeding strategy is currently available. Of particular interest is whether hybrid vigour exists and can be exploited in breeding programmes. While there are reports of hybrid vigour in interspecific hybrids of *Eucalyptus* (e.g. Boden 1964; Venkatesh and Sharma 1977a; Venkatesh and Sharma 1977b), intraspecific controls are usually absent or insufficient (e.g. open pollinations) and it is difficult to assess whether similar genetic gain could be achieved simply through the removal of inbreeding by wide intraspecific outcrossing. Superiority of interspecific hybrids may result from true heterosis (dependent on non-additive gene effects) or, as is more likely the case, be due to the beneficial combination of complementary traits (dependent on additive gene effects) (Martin 1989; Nikles and Griffin 1991). In the latter case, hybridisation of species with complementary traits may result in synergistic effects in specific environments where neither of the species is well adapted (Sedgley and Griffin 1989; Martin 1989). Such a case, for example, is the *E. grandis* x *urophylla* hybrid used in Brazil, where the hybrid gains resistance to stem canker from *E. urophylla* and fast growth and good form from *E. grandis* (Campinhos and Ikemori 1989).

2 Reproductive Barriers

A detailed knowledge of crossability patterns is essential as reproductive barriers may preclude many otherwise desirable species combinations from direct commercial exploitation. At the pre-zygotic level, general trends have been noted in the genus (see Pryor 1976; Griffin *et al.* 1988; Ellis *et al.* 1991). The major subgenera are reproductively isolated (Pryor 1976; Griffin *et al.* 1988), as physiological barriers result in most pollen tube growth being arrested in the upper

style (Ellis *et al.* 1991). However hybridisation within subgenera is relatively common (Pryor 1976; Griffin *et al.* 1988) and, in many cases, controlled pollinations amongst closely related species (within Section) have demonstrated few post-mating, pre-zygotic barriers to seedset (Cauvin 1983; Potts *et al.* 1987; Tibbits 1989). Nevertheless, a major pre-zygotic barrier to hybridisation within subgenera has been identified which is structural and unilateral and is due to the pollen tubes of small flowered species being unable to grow the full length of the style of large flowered species (e.g. Potts and Savva 1989; Gore *et al.* 1990). In addition, there is mounting evidence to suggest that both pre-zygotic (Pryor 1957a; Ellis *et al.* 1991) and post-zygotic (e.g. Pryor 1957a, 1976; Pilipenka 1969; Potts *et al.* 1987; Griffin *et al.* 1988) barriers to crossing within subgenera increase with increasing taxonomic distance between species. An optimum degree of genetic/taxonomic divergence for cross success and the expression of hybrid vigour has been hypothesized (e.g. Potts *et al.* 1987; Martin 1987) resulting from the expression of inbreeding depression at one extreme and outbreeding depression in wide interspecific crosses at the other.

3 Case studies

Several recent studies undertaken in Tasmania have attempted to compare the success of hybridisation with different levels of inbreeding within the parental species. Wide intraspecific outcrosses of either species, usually inter-provenance crosses, have been used as controls to assess the effects of interspecific hybridisation. When the effects of inbreeding within the parental species are removed, these studies have revealed marked pre- and post-zygotic barriers to interspecific hybridisation, even between relatively closely related species.

E. gunnii x *globulus*

Hybridisation of *E. gunnii* and *E. globulus* (often treated as a subspecies - *E. globulus* subsp. *globulus* [Chippendale 1988]) is of particular interest as this would allow the combination of genes of one of the most freezing-resistant species in the genus with genes of one of the faster growing, high pulp yielding species (Pryor 1957b; Cauvin *et al.* 1987; Potts *et al.* 1987; Orme and Hetherington 1991). These two species are grouped in the same *Symphyomyrtus* series (Series *Viminales*; Section *Maidenaria*) by Pryor and Johnson (1971). Earlier work demonstrated that the F₁ hybrid is relatively vigorous (Potts unpubl. data) with freezing-resistance intermediate, but with slight partial dominance toward *E. globulus* (Tibbits *et al.* 1991). Previous crosses undertaken using the larger flowered species, *E. globulus*, as the female parent virtually all failed (Potts and Savva 1989; Gore *et al.* 1990; Fig. 1) whereas several crosses undertaken using two *E. gunnii* females in a natural stand at Snug Plains were successful (Potts and Cauvin 1988). These results were the stimulus for further investigation of this hybrid combination and in 1990, 14 unselected *E. gunnii* females growing in three natural stands on the Central Plateau of Tasmania were crossed in a factorial (NC II) design

with 8 *E. globulus* pollen parents selected by APPM Forest Products on the basis of growth rate and pulp yield. One intra- and two inter-provenance outcrosses of *E. gunnii* were undertaken on each female as well as an unassisted self-pollination. Single-pair unrelated matings involving the 8 *E. globulus* parents were provided by APPM as the outcross controls for *E. globulus*.

The results from this crossing programme indicated marked pre- and post-zygotic barriers to the

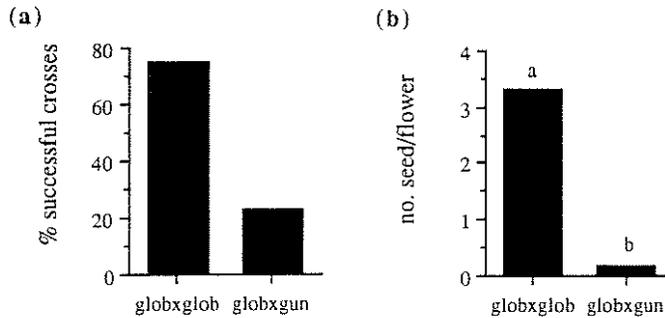


Fig. 1. The success of wide intraspecific (globxglob; n= 20) and interspecific *E. globulus* x *gunnii* F1 hybrid (globxgun; n= 13) crosses using *E. globulus* as the female parent. The percentage of successful crosses (a) and the mean number of seed obtained per flower pollinated (b) are indicated. Common letters indicate groups which are not significantly different at the $P < 0.05$ level.



Fig. 2. The success of self (n=10), open-pollinated (n=15), intraspecific (gunxgun; n= 36) and interspecific *E. gunnii* x *globulus* F1 hybrid (gunxglob; n=76) crosses using *E. gunnii* as the female parent. The percentage of successful crosses (a) and the mean number of seed obtained per flower pollinated (b) are indicated. A cross was classified as unsuccessful if no viable seed was obtained. Common letters indicate groups which are not significantly different at the $P < 0.05$ level.

production of the *E. gunnii* x *globulus* F₁ hybrid, even when the smaller flowered species is used as the female parent. Only 74% of the *E. gunnii* x *globulus* crosses were successful compared with 93% of the intraspecific controlled crosses on the same *E. gunnii* females (Fig. 2a). Capsule set, and the number of viable seed obtained per capsule, from the hybrid crosses was 47% (20.3 versus 43.6% capsules/flower crossed; P < 0.001) and 36% (3.3 vs 9.1 seed/capsule; P < 0.001), respectively, of that obtained in the intraspecific outcrosses. This resulted in the total number of viable seed obtained per flower being reduced to only 16% of the intraspecific outcrosses (0.58 vs 3.53 seed/flower; P < 0.001; Fig. 2b). The barrier to the production of the *E. gunnii* x *globulus* F₁ hybrids was further accentuated by poor survival of the hybrid germinants when transferred from their agar (t.c.) germination medium (Fig. 3).

These pollinations were undertaken on *E. gunnii* females growing in high-altitude, natural stands and, while significant differences in cross success were observed between localities, the interaction between cross-type and locality was insignificant for all variables. Nevertheless, it is possible that the yield of hybrid seed may differentially increase in warmer, low-altitude, exotic environments because, for example, species may have different optimal temperatures for germination and pollen tube growth (Potts and Buchanan unpubl.).

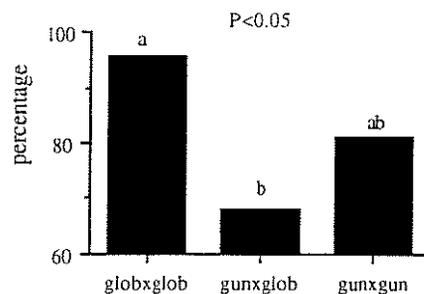


Fig. 3. The percentage survival of germinants from *E. globulus* (globxglob; no. families =15) and *E. gunnii* (gunxgun; n=17) intraspecific families and interspecific *E. gunnii* x *globulus* F₁ hybrid (gunxglob; n=23) families. Data was arc-sine transformed for analysis and common letters indicate groups which are not significantly different at the P < 0.05 level. (Data provided by W. Tibbits, APPM Forest Products)

E. nitens x *globulus*

E. nitens and *E. globulus* are the two most important forestry species in south-eastern Australia (Volker and Raymond 1989; de Little *et al.* 1992). The two species are closely related and are grouped in the same subseries (Subgenus *Symphyomyrtus*, Series *Viminales*, Subseries *Globulinae*) (Pryor and Johnson 1971). Commercial interest in hybridisation between these two species lies in the potential to combine the fast growth, coppicing ability, resistance to specific insect pests and high pulp yields of *Eucalyptus globulus* with the fast growth and better freezing-

resistance of *Eucalyptus nitens* and to eliminate negative traits such as *E. globulus*' susceptibility to *Mycosphaerella* spp. leaf spot fungi and the poor branch shedding and rooting abilities of *E. nitens* (Volker and Raymond 1989; de Little *et al.* 1992). However, there are difficulties as *E. nitens* and *E. globulus* differ markedly in flower size and the F₁ hybrid can only be produced using the smaller flowered *E. nitens* as the maternal parent due to the style of *E. globulus* being too long for the short pollen tube of *E. nitens* to reach the ovary (Gore *et al.* 1990). There is considerable interest in breaking this unilateral barrier as the *E. globulus* flower is relatively large, is easy to emasculate and pollinate, and has the potential to produce larger and greater numbers of seed than an *E. nitens* flower. However to date, treatments such as artificially shortening the *E. globulus* style have been unsuccessful (Gore *et al.* 1990).

Following the initial success of a small scale planting of hybrids between these two species (Tibbitts 1988, 1989), a large crossing programme between and within these two species was undertaken by the CSIRO. The crossing design involved an 8 x 26 factorial within *E. globulus*, a 10 x 10 half-diallel amongst *E. nitens* parents from the Toorongoo provenance, and a 6 x 14 *E. nitens* x *globulus* factorial. Open-pollinated seed was also obtained from all parents and females were all self-pollinated (unassisted and assisted). The major *E. globulus* factorial was partitioned into smaller inter- and intra-provenance factorials involving trees from the Bass Strait Islands (King Island and Flinders Island) and the more southern Taranna provenance, resulting in four different levels of inbreeding within *E. globulus* (selfs, o.p.'s, intra- and inter-provenance crosses; see Volker and Orme [1988] for provenance details). The same parents used in the interspecific factorial were used in the intraspecific crosses to allow direct comparison of genetic parameters. Progenies from these crosses along with several controlled crosses of *E. bicostata* (often treated as a subspecies of *E. globulus*) and its F₁ hybrid with *E. globulus* were planted on several sites in 1990 using a modified α -lattice design (4 reps of 5 tree plots per family per site) (Patterson and Williams 1976). However, due to limited seed availability, most of the hybrid families were planted on only a single site at West Ridgley, Tasmania. Families in this trial were assessed in the nursery for the frequency of deleterious abnormalities and after one years' plantation growth for 'severe' and 'potentially lethal' abnormalities and mortality.

There were marked pre- and post-zygotic barriers to both the production of the *E. nitens* x *globulus* F₁ hybrids and inbreeding in both species. Seed was obtained from only 37% and 56% of the self-pollinations of *E. globulus* and *E. nitens*, respectively, whereas over 90% of the intraspecific outcrosses were successful (Fig. 4a). All of the *E. nitens* x *nitens* crosses attempted were successful to some extent, whereas seed was obtained from only 81% of the hybrid crosses undertaken on the same females. Capsule set and the number of viable seed obtained per capsule from the hybrid crosses was 69% (21.7 versus 31.2% capsules/flower crossed; P > 0.05) and 50% (3.2 vs 6.5 seed/capsule; P < 0.01), respectively, of that obtained in the intraspecific

outcrosses. This resulted in the total number of viable seed obtained per flower in hybrid crosses being only 35% of that obtained in the intraspecific outcrosses (0.68 vs 1.95 seed/flower; $P < 0.01$; Fig. 4b), which is significantly greater than the 2.6% for unassisted self-pollination (0.05 seed/flower). There was no significant relationship between the average success of the male parents in pure species compared with hybrid combination, as measured by either capsule set (cap/fl $r = 0.32$ n.s.; d.f. = 24), seed set per flower crossed (sd/fl $r = 0.28$ n.s.) or per capsule collected (sd/cap $r = 0.22$ n.s.). Thus, male success in pure species crosses cannot be used as a predictor of cross success in hybrid combination.

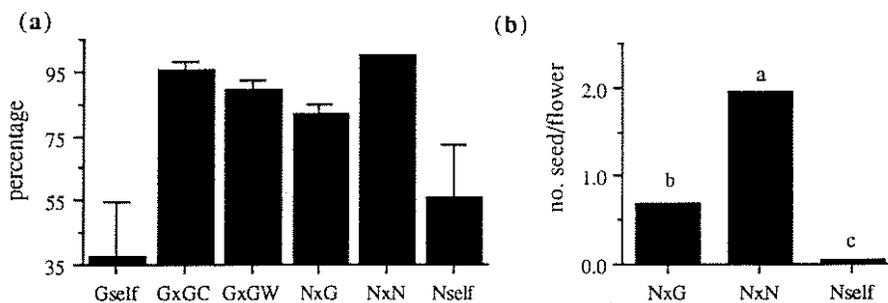


Fig. 4. The success of self ($n=8$), intraspecific ($N \times N$; $n=45$) and interspecific *E. nitens* \times *globulus* F1 hybrid ($N \times G$; $n=124$) crosses using *E. nitens* (N) as the female parent and self ($n=8$), intraprogenance (GxGC; $n=91$) and interprogenance (GxGW; $n=114$) crosses using *E. globulus* (G) as the female. Histograms indicate (a) the percentage of genetically different crosses in which at least some viable seed was obtained and (b) the number of viable seed obtained per flower pollinated. Common letters indicate groups which are not significantly different at the $P < 0.05$ level.

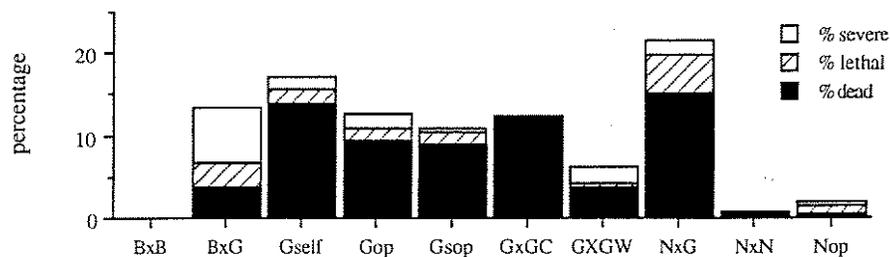


Fig. 5. The percentage mortality and of severe or potentially lethal abnormalities recorded after 1 years' plantation growth. Percentages are maximum likelihood estimates derived from a logit model (CATMOD, SAS). Codes are detailed in Table 1.

Table 1. The success of *E. nitens* and *E. globulus* crosses in the nursery and after 1 years' plantation growth

The table indicates the number of families assessed (nursery) and in which deleterious abnormalities or abnormalities and mortalities were recorded in the nursery and plantation respectively, the number of individuals assessed and the percentage abnormal in the nursery and abnormal and dead in the plantation.

(¹ includes mortalities plus severe and potentially lethal abnormalities in the plantation)

Cross type	Families			Individuals			
	N	% with abnormalities ¹		N	Nursery % abnormal	Plantation	
		Nursery	Plantation			N	% abnormal ¹ + dead
<i>E. bicostata</i> (BxB)	4	0.0	0.0	398	0.0	95	0.0
<i>E. bicostata</i> x <i>globulus</i> (BxG)	6	66.7	83.3	108	18.5	104	13.5
<i>E. globulus</i>							
- self (Gself)	3	33.3	33.3	64	1.6	58	17.2
- o.p. natural stand (Gop)	25	16.0	72.0	645	1.2	491	12.6
- o.p. seed orchard (Gsop)	8	37.5	50.0	309	2.5	155	11.0
- intraprovenance (GxGC)	84	3.6	53.0	1913	0.2	1641	12.5
- interprovenance (GxGW)	86	16.2	49.4	2085	3.2	1809	6.1
<i>E. nitens</i> x <i>globulus</i> F1 (NxG)	43	37.2	76.7	690	12.9	643	21.6
<i>E. nitens</i> - intraprovenance (NxN)	36	8.3	13.9	869	0.8	703	0.7
- o.p. seed orchard (Nop)	10	10.0	20.0	1298	0.1	210	1.9

The barrier to the production of the *E. nitens* x *globulus* F1 hybrids was further accentuated by high levels of abnormalities and differential mortality in early stages of the life cycle. The deleterious effects of hybridisation were manifest in the nursery but inbreeding effects only became evident after 1 years' plantation growth (Table 1 ; Fig. 5). Hybrids of *E. globulus* with both *E. bicostata* and *E. nitens* had significantly ($P < 0.001$) higher levels of abnormalities in the nursery than any of the pure species crosses. 13% of the *E. globulus* x *nitens* F1 hybrids were recorded as having deleterious abnormalities in the nursery and integrating mortality, this increased to 22% after one years' plantation growth. In contrast, mortality and deleterious

abnormalities after one year accounted for a loss of only 6% and 1% of the of *E. globulus* (interprovenance) and *E. nitens* outcrosses, respectively. The highest levels of abnormal seedlings in the nursery occurred in the 6 *E. bicostata* x *globulus* F1 families. However, losses in the field were comparable to that observed with high levels of inbreeding in *E. globulus*, although still significantly ($P < 0.01$) greater than in interprovenance crosses of *E. globulus* and the *E. bicostata* outcrosses. Losses between germination and planting out in the nursery were not assessed, therefore the magnitude of early post-zygotic selection may be underestimated. The expression of deleterious abnormalities in specific hybrid families was not predictable on the basis of the levels of abnormalities in pure species combinations which, coupled with the fact that the deleterious abnormalities are expressed at an earlier stage in the life cycle, suggests that they result from a different genetic mechanism to that resulting in inbreeding depression.

4 Discussion

A major limitation to the exploitation of intraspecific outcrosses and interspecific hybrids amongst the cool temperate species examined is clearly the ability to economically mass produce propagules. Differences in flowering time within and amongst these species and differences in flower morphology necessitates the use of controlled pollination (Volker *et al.* 1988). In the closely related species examined, hybrid seed production is 16-35% less than that of intraspecific outcrosses, and deleterious abnormalities and mortality in hybrid progenies may be over three to four times higher. At least some of the selective cost of these deleterious abnormalities can be reduced by roguing in the nursery, however, this does not appear to be the case with inbreeding where the post-zygotic effects seem to manifest later in the life cycle. Wide crossing within both *E. nitens* and *E. globulus* minimised the level of deleterious genetic effects and this optimum is also reflected in the early growth rate of plants of normal phenotype (data not shown). These early results suggest that adoption of breeding strategies which exploit wide intraspecific crossing may avoid many of these problems. Nevertheless, despite high levels of abnormalities and mortality in many of the *E. globulus* x *nitens* hybrid families, the early vigour of survivors of normal phenotype was comparable to that of intraspecific outcrosses and, with F1 hybrids generally intermediate in frost resistance (Tibbitts *et al.* 1991), there is still the potential for selection of productive hybrid genotypes. However, where complementarity is being exploited, the relative performance of hybrids will be environmentally dependent (e.g. Martin 1989), necessitating testing of hybrids and parental controls across a range of environments. Clonal multiplication prior to field testing would thus be advantageous where controlled cross seed is limited and this strategy is currently being adopted with the *E. gunnii* x *globulus* crosses.

The commercial exploitation of hybrid selections will clearly depend on the concurrent development of techniques for clonal propagation. Controlled pollination of *E. nitens*, for

example, is extremely time consuming and, with relatively low seed yields even with intraspecific crosses, the operational production of hybrid seed is not practical. While subtropical and tropical species and their hybrids are readily vegetatively propagated by cuttings (e.g. Marien 1991), this is not the case for the cool temperate eucalypt species such as *E. globulus* and *E. nitens* for which micropropagation techniques are still being developed (de Little *et al.* 1992). Nevertheless, improved cloning potential may be a by-product of hybridisation with species which are more readily propagated from cuttings. The early expression of deleterious genetic effects in the hybrids examined means that seedlings can be beneficially screened prior to cloning. However, cloning directly from seed is often used to circumvent the problem of obtaining sterile vegetative material for use with micropropagation techniques (e.g. Willyams *et al.* 1992). In the case of interspecific hybrids, our data would suggest that such precocious cloning may incorporate a large percentage of deleterious abnormalities. This problem would not be as severe when establishing clones or mother plants from more advanced seedlings and would be further reduced by the use of more advanced material, although problems with juvenility and sterility then arise.

Acknowledgements

The *E. nitens* x *globulus* crossing programme was instigated by Dr. A. R. Griffin and undertaken in 1987-1989 by the CSIRO Division of Forestry (P. Volker) with assistance from the Australian Special Rural Research Fund. Both hybridisation projects were supported by APPM Forest Products.

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