

# *Corymbia* Research Meeting

Underpinning development of a profitable hardwood plantation industry in northern Australia by research into *Corymbia* species and hybrids.



Inaugural meeting of *Corymbia* researchers and the plantation industry.

**Gympie, Queensland 1<sup>st</sup> and 2<sup>nd</sup> June 2005**

Convened by David Lee, DPI&F

Chaired by Mark Lewty, DPI&F

Corymbia Research Meeting: Underpinning development of a profitable hardwood plantation industry in northern Australia by research into Corymbia species and hybrids.

The Department of Primary Industries and Fisheries (DPI&F) seeks to maximise the economic potential of Queensland's primary industries on a sustainable basis.

This publication has been compiled by David Lee, Department of Primary Industries and Fisheries, Delivery, Horticulture and Forestry Science.

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We would like to acknowledge the provision of the bus for the field trip and lunch on day one of the meeting by:



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## **CORYMBIA SPECIES AND HYBRIDS: A SOLUTION TO QUEENSLAND HARDWOOD PLANTATIONS?**

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### **INTRODUCTION**

Hardwood plantations are relatively new in northern Australia. Of the species available, the spotted gums are the most important commercial hardwood plantation taxon for high quality timber in the humid and sub-humid zones of Queensland and northern New South Wales. The domestication and genetic improvement of the spotted gums (*Corymbia citriodora* subsp. *citriodora* (CCC), *C. citriodora* subsp. *variegata* (CCV) and *C. henryi* (CH) however only commenced in Queensland in 1997. Since then seed orchards and progeny trials have been established for all three species. One major failing of these species is all provenances are somewhat susceptible (Dickinson *et al* 2004) to the fungal disease, Ramularia Shoot Blight caused by *Quambalaria pitereka* (Simpson 2000). In amenity plantings and wind breaks of *C. torelliana*, hybrids with the spotted gum species were noticed that had great vigour, good form and resistance to Ramularia Shoot Blight. As a consequence a breeding program has been initiated in Queensland, to hybridise the spotted gum species with *C. torelliana* (CT). This paper reports on the genetic base of each species and presents preliminary result on the performance of these species and hybrids in trials.

### **THE CORYMBIA GENETIC BASE IN QUEENSLAND**

A broad genetic base has been developed for the spotted gum species to underpin the long term genetic improvement of the three species that occur in northern Australia (Table 1). The fourth species in the spotted gum complex *C. maculata* has not been included in the program to date due to it's know high level of susceptibility to Ramularia Shoot Blight. A broad genetic base has also been established for *C. torelliana* to underpin further breeding and genetic development of the Corymbia hybrids.

**Table 1.** Genetic base of *Corymbia citriodora* subsp. *citriodora* (CCC), *C. citriodora* subsp. *variegata* (CCV) *C. henryi* (CH) and *C. torelliana* (CT) in seed orchards and progeny trials in Queensland. Ramularia Shoot Blight tolerance or resistance is indicated

Species	Number progeny / seed orchards	Number provenances	Number seed parents (nominally)	Ramularia tolerant provenances identified <sup>1</sup>
CCC	1	17	700	Yes
CCV	8 <sup>2</sup>	44	656 (+27 <sup>3</sup> )	Yes
CH	1	7	47 (+29 <sup>4</sup> )	No
CT	3	6	113	All field resistant

<sup>1</sup> For CCC, CCV and CH, more Ramularia tolerant provenances are detailed in Dickinson *et al* 2004

<sup>2</sup> Trials range from Gatton, Gympie –Tiaro region, Miriam Vale and the southern Burnett.

<sup>3</sup> Four additional provenances are planted in taxa trials with an additional 27 seed parents.

<sup>4</sup> Three more provenances are represented in taxa trials with an additional 29 seed parents.

## RESULTS AND DISCUSSION

### *Corymbia citriodora subsp. variegata*

CCV height growth and Ramularia shoot blight were found to be highly correlated ( $R^2 = 0.83$ ), with the average height growth at 2.8 years being 5.9 m across five progeny trials. These five progeny trials contain a subset (25 provenances and 319 half sib family seedlots) of the genetic base now planted for the species and all focus on high rainfall environments 900+ mm MAR. With the hardwood plantation industry transitioning to more marginal environments it is considered that this genetic base will need expansion to include CCV provenances from lower rainfall environments. This is now being address with the recent planting of progeny trial in the Burnett with further targeted CCV progeny / provenance collections planned. The progeny trial results support Dickinson's *et al.* (2004) findings that Woondum provenance (from the Gympie region) is generally better in terms of Ramularia Shoot Blight tolerance and height growth than lower rainfall provenances, however, in these progeny trials Richmond Range, Grange and additional Gympie region provenances perform as well as the Woondum source.

### *Corymbia citriodora subsp. citriodora and Corymbia henryi*

Although the genetic base for both CCC and CH is broad, only one seed orchard / progeny trial has been established for each species. To ensure the security of the genetic base of these species, it is essential that further trials testing new environments be established. This will ensure the genetic bases are secure form catastrophic loss such as fire, pest and disease, etc. It is unlikely these species will be planted on a commercial basis however they will form a basis for the further development of the *Corymbia* hybrids.

### *Corymbia hybrids*

Since 2000 a breeding program has been underway to evaluate the potential of hybrids between *C. torelliana* and the spotted gum species (CCC, CCV, CH and CM). The resulting *Corymbia* hybrids are promising across all sites where they have been planted. Currently progeny trials of the hybrids have been planted across 16 sites (46 hectare of progeny trials) ranging from Walkamin (Queensland) to Grafton (New South Wales). In these trials, the hybrids are exhibiting a range of beneficial growth and performance traits (Table 2), relative to their parental species including:

- Ramularia Shoot Blight tolerance,
- frost resistance,
- amenability to propagation as rooted cuttings,
- hybrid vigour for growth (height and diameter).

### *Commercial deployment of Corymbia species and hybrids*

The spotted gum species (CCC, CCV and CH) are not amenable to propagation as rooted cuttings; hence the only practical option to deploy improved germplasm from the breeding program in routine plantations is via seed. However, in the seed orchards wide spread synchronous flowering has not occurred in the last five years. This along with the susceptibility of all species of spotted gum to Ramularia Shoot Blight has lead to a switch away from developing the species for operational development to developing the *Corymbia* hybrids for operational deployment. These hybrids combine the best attributes of the parental species and are amenable to propagation as rooted cuttings.

In three-year-old *Corymbia* hybrid progeny trials, a number of outstanding trees have been selected, based on growth, pest and disease tolerance and form attributes. Currently eight of these selects have been captured as cuttings to commence a clonal testing and deployment program. These hybrids represent the first release from the five-year-old breeding program

and are the best individual trees from five families. They have not been tested as rooted cuttings in clonal tests. Clonal tests are about to be initiated and their results will determine additional clones for commercial propagation in the future. The rolling front breeding program uses controlled crossings between known parents of *Corymbia torelliana* and *Corymbia citriodora* subsp. *variegata* to generate improved hybrids. Hybridisation and clonal testing in preparation for commercial release is anticipated to continue over the next 20 years. Part of the breeding program aims to introduce parental varieties currently not under test to improve the growth, frost and pest and disease tolerances of *Corymbia* hybrids. This work forms part of an integrated program looking at all aspects of growth, silviculture and management of *Corymbia* hardwood plantations.

**Table 2.** Relative performance levels of the parent species and hybrids based on observations in trials at Amamoor Dam and Coolabunia. Taxon included are *C. torelliana* (CT), *C. citriodora* subsp. *variegata* (CCV) and the hybrid between these two species (F1 hybrid).

Trait × Taxon	CT Kuranda <sup>5</sup>	CCV Woondum <sup>5</sup>	F1 hybrid
Ramularia tolerance	Very high (immune?)	Moderate	High +
Erinose mite tolerance	Very high (immune?)	Very low	High +
Red Shouldered Leaf Beetle tolerance	Low	High	High
Longicorn tolerance	Moderate	High	Moderate
Swarming scarabs tolerance	High	Low	High
Sooty mould tolerance	Low	High	Moderate
<i>Mycosphaerella</i> tolerance	High	High	Moderate
Height and diameter growth	Low to moderate	Low to high	Low to very high
Straightness level	Moderate	Moderate	High
Taper (low desirable)	High	Low	Moderate
Branch quality, including shedding	Low	High	Moderate to high
Canopy density (high results in good site capture)	High	Low to moderate	High
Per cent abnormal seedlings	Very low	Very low	Low to high variable by family
Cold, drought hardiness	High	Low to moderate	High
Environmental plasticity	High	Moderate	High
Seedling / coppice rootability	Moderate	Very low	Moderate

Note: Giant wood moth does not attack *Corymbia*

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#### ACKNOWLEDGEMENTS

We would like to acknowledge the contribution of many staff at DPI&F Horticulture and Forestry Science and the provision of land for trials from DPI-Forestry.

<sup>5</sup> Relative level may vary depending on family and provenance.

# GENETIC DIVERSITY AND CROSS-COMPATIBILITY OF CADAGI (*Corymbia torelliana*): IMPLICATIONS FOR HYBRID BREEDING

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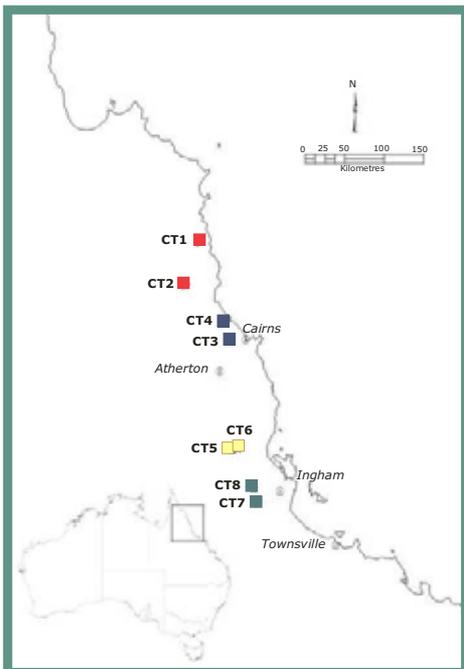
## BACKGROUND

Cadagi's (*Corymbia torelliana*) natural distribution is restricted to the rainforest margins in North Queensland between Ingham and Cooktown, however, it has been widely planted as an ornamental and in amenity plantings, particularly in southeast Queensland. Hybrids with spotted gum taxa show great potential for hardwood plantation forestry in northern Australia, particularly in marginal rainfall areas.

## GENETIC DIVERSITY

To assess the level of genetic diversity within and between the populations, leaf material from sixteen individuals from each of eight populations of *C. torelliana* were sampled from across the species' geographic range (Figure 1). In addition, 37 *C. torelliana* individuals from amenity plantings around southeast Queensland, that have been used in controlled pollinations with spotted gum taxa, were sampled in order to identify the most likely origin of this material and the level of genetic diversity captured in the breeding program.

Genomic DNA was extracted from all individuals using Qiagen's Plant DNeasy Mini Kit. Six microsatellite markers developed in *C. citriodora* subsp. *variegata* (Jones *et al.* 2001) and a chloroplast DNA locus (JL<sub>A</sub>+; Freeman *et al.* 2001) were utilised in order to understand both contemporary and historical relationships between populations of *C. torelliana*.

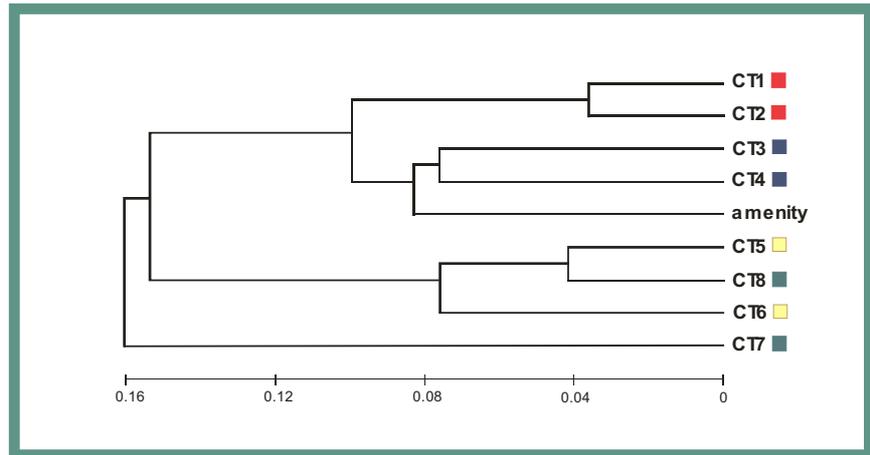


**Figure 1:** Location of sampled *C. torelliana* populations

The microsatellite data revealed a high level of genetic diversity within the natural *C. torelliana* populations, with overall expected and observed heterozygosity being 0.808 and 0.766 respectively. The amenity trees also had high levels of heterozygosity ( $H_E=0.832$  and  $H_O=0.788$ ). Inbreeding levels (overall  $F=0.058$ ) were not significant in *C. torelliana* except for the two northern populations (CT1&2).

Although only 5.5% of total genetic variation found using microsatellites was between the natural *C. torelliana* populations, it was geographically structured (Figure 2). A similar pattern of variation was also found using chloroplast DNA (data not shown). The amenity trees were more closely related to *C. torelliana* populations, CT3 & 4, however, individual assignment tests are required to confirm this.

**Figure 2:** UPGMA phenogram of Nei's 1978 genetic distance between *C. torelliana* populations and amenity trees



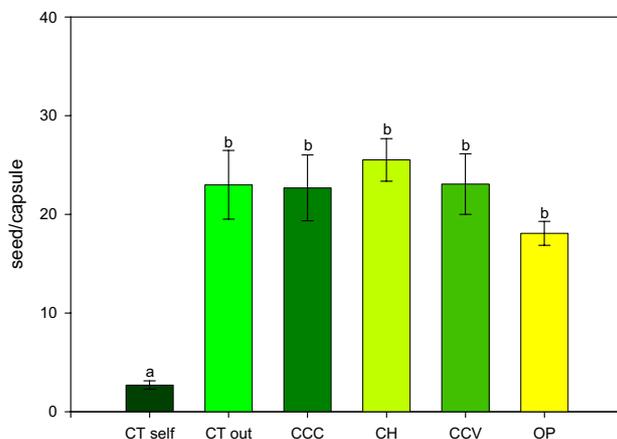
### CROSS-COMPATIBILITY

To examine the cross-compatibility of *C. torelliana* (CT) with spotted gum taxa, controlled pollinations were undertaken using a replicated block design over two flowering seasons. In 2002, eight CT amenity trees were chosen and five pollen treatments (CT self, CT outcross, CCC, CCV, CH) were applied in three replicates of 50 flowers to each tree. Open-pollinated capsules were also collected from the CT trees in the 2002 season. In 2003, nine CT amenity trees were pollinated with three replicates of 35 flowers using five pollen treatments (CT self, CT outcross, CCC, CCV and CX). For each pollen treatment and flowering season, capsule retention, seed weight, total seed yield and seed/capsules were calculated (eg. Figure 3). Preliminary results suggest that pollinations with the spotted gum taxa examined were comparable to those pollinated with outcross CT pollen. Seed from four CT trees per season was tested for germination and the height of seedlings measured prior to planting.

### IMPLICATIONS FOR HYBRID BREEDING

The majority of the genetic variation in *C. torelliana* has been captured in the amenity trees used in creating hybrids with spotted gums. The origin of the amenity trees is most likely to be the Kuranda region. Broadening the genetic base of the breeding population should be targeted towards collection from populations at the southern extremity of *C. torelliana*'s natural range.

Further analysis will provide greater insight into the attrition rates inherent in creating F<sub>1</sub> hybrids. The controlled pollinations undertaken will also allow selection of parents with good breeding values and provide outstanding families and individuals to target for deployment.



**Figure 3:** Average seed produced per capsule in 2002 crosses on CT trees (n=8) with different pollen parents.

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# **CORYMBIA TORELLIANA HYBRIDS FOR HARDWOOD FORESTRY- WITHOUT THE WEEDINESS!**

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## **INTRODUCTION**

*Corymbia torelliana* hybrids have great potential for sustainable plantation forestry in many areas of tropical Australia. The species as a parent in hybrid breeding programs confers benefits such as resistance to *Ramularia* shoot blight, environmental plasticity, effective site capture and good rooting ability for clonal forestry.

*C. torelliana* occurs naturally in rainforests and rainforest margins in the wet tropics region of Far North Qld between Shiptons' Flat, near Cooktown and Mt Fox, near Ingham. The species has a tendency to become a weed where it is planted in areas outside of the wet tropics. Consequently, 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 may contribute to its weediness in areas where it has been introduced. Seeds are dispersed by bees, sometimes up to 300 m from the parent tree (Wallace and Trueman 1995). Native stingless bees of the genus *Trigona* build their nests from plant resins, and *T. carbonaria* forages 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 nests. Seeds dispersed by bees are almost all viable, and abundant germination and establishment occurs 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*, when used in nest structures, tends to collapse, causing death of the colony. In spite of no scientific study on the weediness of *C. torelliana* or the effect on stingless bees, *C. torelliana* has been banned from new plantings and actively removed by local councils from amenity plantings.

In this study, we examined the interaction between stingless bees and *C. torelliana* and its hybrids in the natural range of *C. torelliana* in the Wet Tropics. Here we report the structure of hybrid capsules and their attractiveness to stingless bees.

## **METHODS**

We examined capsule structure and attractiveness to bees of an amenity planting of *C. torelliana*, *C. citriodora* subsp. *citriodora* and hybrids of the two species near Walkamin, Qld. All hybrids were full sibling F1's, of reproductive age. We examined the structure of the capsules to determine characteristics that may influence seed dispersal by bees. This included whether resin was present, whether the column had collapsed, the external and internal size and length /width ratio of the capsule, the placement of seeds in resin, and the weight of the seeds.

*C. torelliana*, *C. citriodora* subsp. *citriodora* and hybrids were examined for 10 days over 3 fruiting seasons between 2002 and 2005 to determine whether bees foraged for resin on the capsules and whether bees subsequently dispersed seeds. All observations were carried out between 09.30 and 16.30 H during sunny weather conducive to bee activity. Each tree was initially examined to determine the maturity of the capsules. Where trees had capsules showing signs of opening, e.g. mottled green/brown in colour, valves opening, the capsule clusters were examined for 5 minutes for bee activity using Zeiss 8 X 30 binoculars.

Observations on all trees were repeated every 2-4 days over 2-3 weeks in 2004 and 2005. In preliminary observations we found that bees foraged on hybrid capsules that had been chewed and discarded by red tailed black cockatoos. Where trees had capsules that had been discarded on the ground we conducted further observations of bee activity on the ground for a 5 minute period.

## RESULTS AND DISCUSSION

In ten days of observations, there were no bee visits to capsules on trees of any hybrids or to *C. citriodora* subsp. *citriodora*. In contrast, at the same site, there were on average 2.75 bee visits per 5 minutes to the *C. torelliana* capsules (Table 1). Bees frequently visited broken hybrid capsules on the ground to collect resin from the wound tissue (Table 1). Bees were observed dispersing chaff from these capsules but seed dispersal by stingless bees from these capsules was not observed.

**Table 1.** Attractiveness of *C. citriodora* subsp. *citriodora*, *C. torelliana* and hybrids to stingless bees.

Species	Location of capsules	Mean bee visits (se)	No. of trees	No. of obs.
<i>C. citriodora</i> subsp. <i>citriodora</i>	Tree	0	6	45
<i>C. torelliana</i>	Tree	2.75 (0.48)	4	32
Hybrids	Tree	0	25	167
Hybrids	Ground	3.24 (0.74)	7	29

All hybrid capsules contained some resin. However, they did not have the other characteristics of *C. torelliana* that are necessary for bee dispersal. For example, in many cases the central column had not collapsed sufficiently to allow bees to enter the capsules. Seeds were often much larger than those of *C. torelliana* and were not presented in resin inside the capsule.

### KEY FINDINGS:

- **Bees were not attracted to hybrid capsules** at this site unless they had been damaged by red tailed black cockatoos. Seed dispersal of the hybrids by stingless bees at this site was not observed.
- **Hybrid capsule structure did not allow bees to disperse seeds.**
- **Weed risk of *C. torelliana* hybrids can be managed with careful checking.**

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# **AN INTRODUCTION TO ADaPtE: A EUROPEAN UNION - FUNDED PROJECT COMPARING THE STRUCTURE AND DYNAMICS OF NEUTRAL AND ADAPTIVE GENES IN QUEENSLAND'S SPOTTED GUMS.**

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In plants, the dynamic interactions of migration and selection act to establish genetic structure within and between populations of a species. It is this variation and its distribution within and amongst population that ultimately defines the ability of a species to respond to a changing environment and shifting selection pressures. Traditionally genetic diversity on forest tree species is measured at molecular markers which are neutral. There is however concern among forest scientists that the extent of genetic variation at such markers does not reflect that of adaptive traits and therefore may be of little value for genetic resource improvement and forest management.

Indeed, the description of genetic variation encoding adaptive traits within natural populations has been problematic. In the past, some well-characterised morphological and phenotypic traits known to confer fitness advantages and to be under single -locus control following Mendelian segregation analysis (e.g. flower colour) have been available. However, such systems tended to be the exception not the rule and, since only a few such characters were available, they defined the extent of the ecological questions that could be posed. The recent scientific revolution in the fields of genomics and transcriptomics has allowed a paradigm shift for the field of ecological genetics, and now these new techniques offer the potential of studying the distribution of genetic variation encoding for ecologically important and relevant traits (Schenk et al. 2000), i.e. those that have evolved in response and are maintained by environmental extremes and selection pressures.

ADaPtE (Adaptive Diversity and Population Structure in Eucalypts) is a recently started EU-funded project which therefore aims at producing a fine scale map of the distribution of neutral and adaptive gene variation in spotted gum populations distributed across a steep cline of water availability in central Queensland by 1) Quantifying neutral genetic structure and dispersal parameters using available microsatellite markers and well characterised population genetics analytical methods. 2) Identifying gene markers for drought tolerance using state-of-the-art functional genomics tools and mapping their distribution across the region. The empirical work will be complemented with simulation modelling to improve our understanding of the role of selection in shaping the distribution of genetic and phenotypic variation across environmental gradients (Bacles et al. 2004).

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## TREE FORM AND THE ROLES OF BORON AND CALCIUM IN HARDWOODS

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A balanced nutrition is essential for the healthy growth and form of tree species. In the absence of the micronutrient boron (B) and/or the macronutrient calcium (Ca) loss of apical dominance occurs rapidly resulting in the onset of poor tree form. Knowledge of specific roles of these elements helps to explain their importance and why tree form deteriorates when their supply becomes limited.

### ROLES OF CALCIUM AND BORON

Calcium and B have essential roles in the binding of pectin in the primary cell wall of all cells within the tree. The primary cell wall is a three dimensional network of: cellulosic microfibrils bundled together as macrofibrils; hemicellulose; and pectin. The pectin is a network of polysaccharide chains bound together with a series of loops. There are three types of polysaccharide chains that make up pectin, namely homogalacturonan, rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII). The homogalacturonan has been referred to as the smooth regions and the latter two as the hairy regions of pectin chains (de Vries *et al.*, 1982; Grant Reid, 1997). The role of Ca in the pectin network is through the formation of Ca-bridges between chains of homogalacturonan. The role of B has been isolated to the formation of a borate-diester bond in the RGII region of the pectin network as a dimer (dRG-II-B) (Kobayashi, *et al.*, 1996; Ishii and Matsunaga, 1996; O'Neill, *et al.*, 1996). These bonds between homogalacturonan and RGII units are thought to be critical for the formation of loops to bind the pectin network with the cellulose microfibrils (Fry, 1986), thereby providing strength and flexibility to the cell wall. Calcium has additional roles in forming Ca-bridges in the middle lamella (between cells) and along with B in stabilising the cell membranes. Boron has a secondary role in the synthesis of lignin for rigidity of the secondary cell wall (Marschner, 1995). Thus in the absence of Ca and/or B, growth of the apical shoots slow or cease due to impaired cell wall development, setting up the tree for a loss of apical dominance.

### SUPPLY OF CALCIUM AND BORON

A number of factors influence the supply of Ca and B for growth. Firstly, Ca and B are passively taken up by the plant, meaning that they are naturally absorbed into roots with the uptake of water and moved around the plant in the xylem. Thus, actively transpiring tissues receive greater amounts of these elements than shoot tips and reproductive structures. They then become trapped in the target tissues with little or no retranslocation to areas of need such as rapidly growing shoot and root tips. There is an exception to this rule for B amongst a small number of species that have evolved with a defacto system of remobilisation of B through cis-diol bonds with sugar alcohols (Brown and Hu, 1996). Therefore in the vast majority of species, disruptions to water uptake and/or nutrients in the soil solution result in rapid effects on cell wall growth in growing tissues such as shoot and root tips.

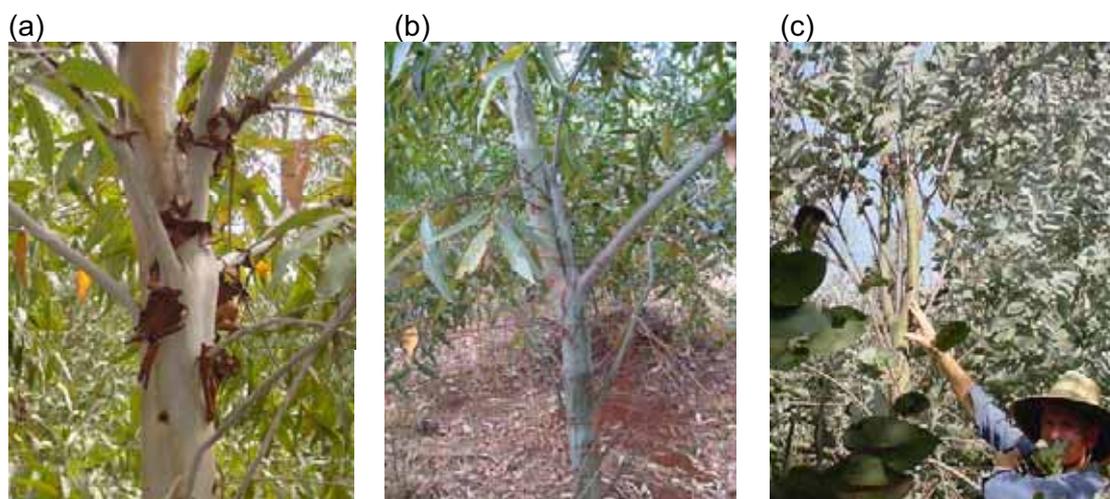
Soil types vary considerably in both B concentrations and their B availability to trees. Soils with sandy loam A horizons (classified as Chromosols, Kurosols and Kandosols under Isbell, 1996) only required 0.4-0.6 mg B kg<sup>-1</sup> Hot CaCl<sub>2</sub> extractable soil B for adequate foliar B in avocados compared to 7.2-11.2 mg B kg<sup>-1</sup> Hot CaCl<sub>2</sub> extractable soil B for Ferrosol soils (as classified under Isbell, 1996) (Smith, 2004). Variations in B supply between soil types are due

to factors such as clay content, sesquioxides (iron and aluminium oxides), organic matter, soil pH and soil water. Thus, soil type differences need to be accounted for when interpreting soil chemical data and determining fertilizer application rates.

## IMPLICATIONS FOR WOOD QUALITY

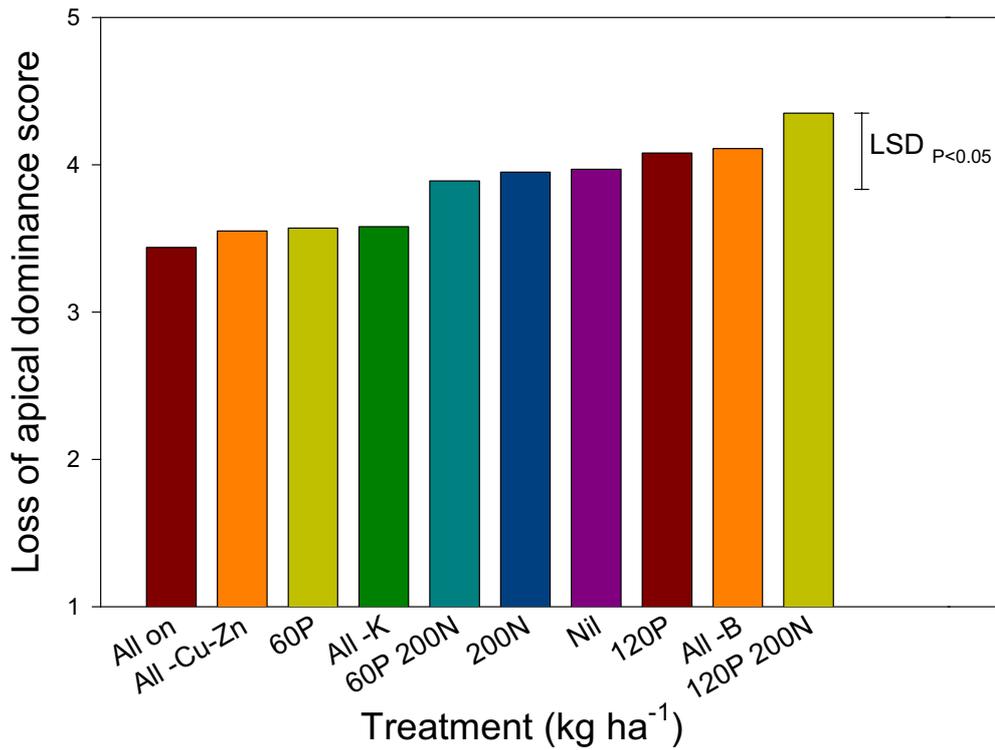
We are not necessarily going to see declines in vegetative tree growth with marginal or temporary deficiencies of these elements as they are not major drivers of metabolic reactions for growth, like elements such as nitrogen and phosphorus. However, declines in tree form and wood quality are the most likely factors to be affected by shortfalls in these elements through direct effects on cell walls. Wood quality assessments are currently being planned to assess the effects of treatments on a range of hardwood species growing in Ca and B field trials.

In our experiments to date there have been -B treatment effects on tree form in *Corymbia citriodora* sub sp. *variegata*, *Eucalyptus pilularis* (Plates 1a & 1b, respectively and Figures 1&2, respectively), and *E. nitens* (Plate 1c) and -Ca and -B treatment effects on tree form of the *Corymbia* complex hybrid (Plate 2).

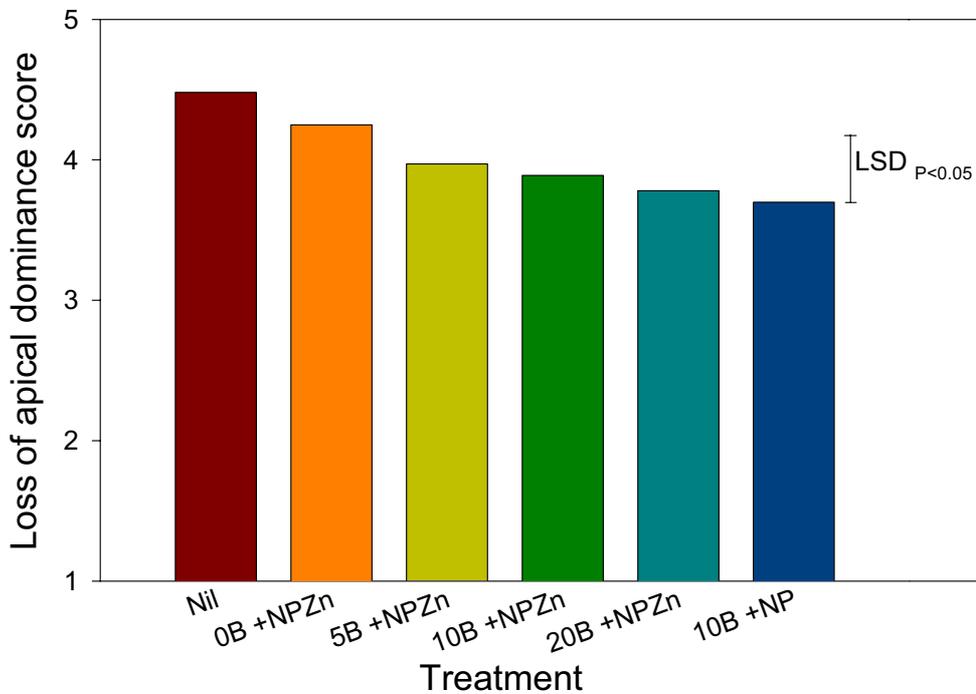


**Plate 1:** Loss of dominance effects on (a) *Corymbia citriodora* sub sp. *variegata* at Tingoora, Qld (b) *E. pilularis* at Blackbutt, Qld and (c) *E. nitens* at Nowendoc, NSW in -B treatments

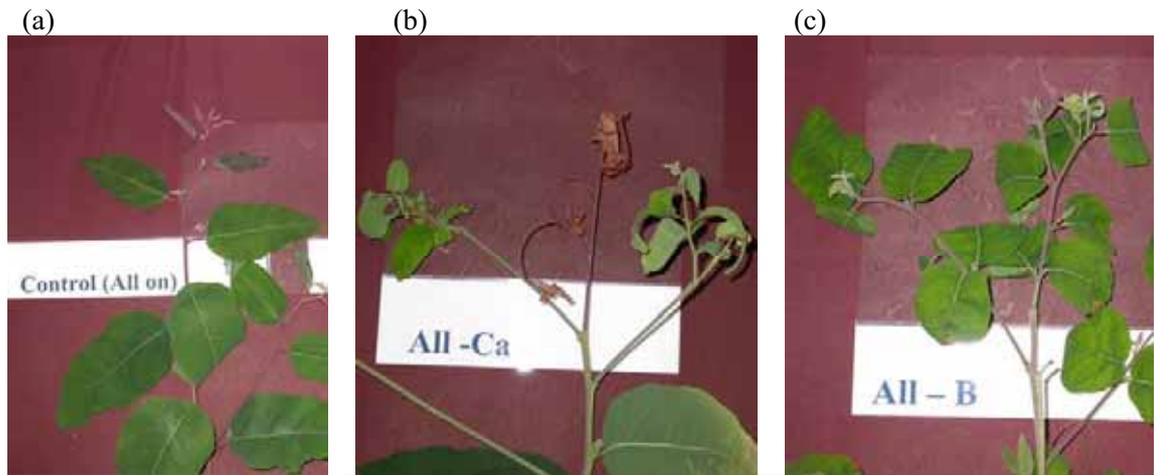
Boron deficiencies are likely in the Queensland hardwood plantations due to low total and available soil B levels along the east coast of Australia, and the greater adsorption of B on Ferrosol soils leading to low plant available B. Unless this issue is addressed through corrective fertilizer additions, then tree form issues are likely to undermine the continued success of these plantations and the value of products at harvest. Similarly, areas of low Ca need further investigation regarding wood quality issues.



**Figure 1:** Loss of apical dominance (LAD) in *Corymbia citriodora* sub sp. *variegata* in low B treatments at age 2 years. Score: 1 = 0 LAD, 5 = Severe LAD



**Figure 2:** Loss of apical dominance (LAD) in *E.pilularis* in low B treatments at age 2 years. Score: 1 = 0 LAD, 5 = Severe LAD



**Plate 2:** Loss of dominance effects in the *Corymbia* complex hybrid grown in (a) complete (All), (b) All –Ca and (c) All –B hydroponic solution treatments at age 5 months

#### ACKNOWLEDGEMENTS

I would like to acknowledge: the financial and staff support of DPIF, in particular the assistance of Paul Keay with experimental work; DPIF Hardwoods and Forest Enterprises Australia for providing land and assistance for field trials; and the assistance of John Huth of DPI&F, Delivery, Horticulture and Forestry Science.

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## INTERSPECIFIC HYBRIDISATION OF *CORYMBIA* SPP.

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This presentation summarises the proposed experimental activities as described in my 'Research Proposal for Confirmed Candidature – Doctor of Philosophy' to be submitted for adjudication with the University of the Sunshine Coast in mid June 2005. I acknowledge the supervision and support from my PhD supervisors Dr. Helen Wallace (SCU) and Dr. David Lee (DPI&F).

The *Corymbia* are a related but phylogenetically separate group from the *Eucalyptus* (Hill and Johnson 1995), yet have had comparatively little research published in the fields of reproductive biology, breeding systems and inter-specific hybridisation. Over the past 10 years, interest in the use of *Corymbia* species for plantation establishment in the tropics and sub-tropics has increased, and this has resulted in the gradual emergence of supporting research programs in these regions (Nikles *et. al.* 2000; Dickinson and Lee 2005). Initial *Corymbia* tree improvement activity focussed primarily on the improvement of pure species lines, with particular selection for disease tolerance to *Quambalaria pitereka* (Dickinson *et. al.* 2004). In more recent times, the great potential of *Corymbia* hybrids as first observed as spontaneous hybrids within pure-species planting, but more recently within DPI&F controlled pollination trials has been recognised, resulting in the initiation of an inter-specific *Corymbia* hybrid program (Lee *et. al.* in press). While existing *Corymbia* hybrids show grow potential, investigations to date have involved hybrid crossing between relatively few parent individuals of only a few *Corymbia* species, with variable success.

The research objectives and activities within this PhD research proposal investigate a range of innovative research questions within the field of *Corymbia* hybridisation. This research will form an important component within the existing DPI&F *Corymbia* hybridisation program and will provide original results to identify the factors which influence the compatibility, incompatibility or incongruity of different *Corymbia* hybrid crosses. It will also improve the understanding of paternal and specific factors which influence the heritability of desirable traits amongst *Corymbia* hybrid progeny. This information will be utilised to develop new methods and strategies to support the DPI&F *Corymbia* hybridisation program, increasing the available genetic resource of useful character combinations and potentially producing superior F1 hybrid and advanced generation hybrid individuals.

The five primary objectives of this Doctor of Philosophy research project are detailed below and will be investigated in a series of five coordinated experiments.

1. To quantify the effects of taxonomy and species relatedness on the hybridising potential of the main *Corymbia* species, sections and related genera.
2. To quantify maternal and paternal effects on the heritability of morphological traits within *Corymbia* hybrids.
3. Identify efficient and effective controlled-pollination techniques for the commercial hybridisation of the *Corymbia* group.
4. To identify the factors which influence the compatibility, incompatibility or incongruity of a range of inter-specific *Corymbia* hybrid crosses.
5. To assess the suitability of a basic range of advanced generation hybrid crosses, to maximise the heritability of desirable traits within progeny.

Experiments investigating objectives 1, 2 and 3 will be initiated in Spring 2005, with experimental methodologies presently well advanced. Experiments investigating objectives 4

and 5 follow-on from the activities in the preceding three experiments and will be initiated in Spring 2006. Hence experimental methodologies for these two experiments are currently in the early development stage. A brief description of the three experiments planned for establishment this year is presented below.

### **EXPT. 1: INTERSPECIFIC HYBRIDISATION WITHIN THE CORYMBIA AND RELATED SPECIES**

Where possible, pollen will be collected or sourced for individual species (3 parents/species), representing six of the seven sections within the *Corymbia* group, as well as one or two individual species from the closely related *Angophora* and *Eucalyptus* genus. As the *Corymbia* sections *Rufaria*, *Politaria* and *Blakearia* contain a greater number of commercial timber species, it is planned to include a greater number of species from these sections in this experiment. Controlled pollinations will be conducted using the one-stop pollination technique with *C. torelliana* as the mother for all treatments. Fertilisation success will be monitored through assessment of % capsule set, seeds/capsule and seed viability. Germinated seedlings will be grown through to age 12 months and will be assessed for growth, survival and seedling morphology.

### **EXPT. 2: PATERNAL EFFECTS ON HERITABILITY OF TRAITS WITHIN HYBRID PROGENY**

This experiment will investigate reciprocal interspecific hybridisation between the two species *C. torelliana* and *C. citriodora* subsp. *variegata*. Reciprocal crosses will be made between a minimum of two unrelated parents of each species. Additional controls of open-pollination, controlled self-pollination and intra-specific pollination will be conducted for each of the four parent individuals. Assessments of fertilisation success and early seedling morphological and growth traits (disease tolerance, clonal propagation) will be conducted for each treatment to an age of 12 months. The results for each hybrid combination will be compared with those measured for pure parent species to quantify the relative influences of paternity or species, on trait heritability.

### **EXPT. 3: CONTROLLED POLLINATION TECHNIQUES FOR THE CORYMBIA**

In recent years controlled pollination operations for eucalypts have been conducted using two main methods; three-stop pollination (Van Wyk 1977) and one-stop pollination or OSP (Harbard *et. al.* 1999). In recent years a third method; artificially induced protogyny (AIP) has been developed which does not involve flower emasculation (Assis *et. al.* 2005). In the limited trials with *Eucalyptus* spp., AIP has proven to be considerably faster than the traditional techniques, achieving similar seed yields, with low contamination levels.

This trial will investigate the efficiency and effectiveness of the AIP technique for a range of inter-specific *Corymbia* hybrid crosses. For each inter-specific hybrid combination, pollination treatments will include the standard 3-stop and OSP methods and the application of the AIP technique on a range of bud maturity classes. The time required for the operator to conduct each pollination technique will be closely monitored. After pollination, measurements of % capsule set, seed viability and contamination levels will be conducted for each treatment. Analysis of these results will determine the efficiency and effectiveness of the AIP technique as compared to the traditional methods, for use in a range of *Corymbia* hybrid crosses

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# EMERGING INSECT PESTS OF CORYMBIA

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## INTRODUCTION

Ramularia shoot blight, caused by the fungus *Quambalaria pitereka*, was the major pest or disease to cause significant losses to spotted and lemon scented gums (CCV and CCC) and other *Corymbia* spp. in SEQ in early plantings. In recent years several new pests such as the eriophyoid mite *Rhombacus* sp., the leaf beetle *Paropsis atomaria* and the bronzing bug *Thaumastocoris* sp. have emerged as important or potential pests of spotted gum and other *Corymbia* spp. Longicorn beetles are also potentially important threats to plantation productivity. The commercialisation of *Corymbia* hybrids and their imminent planting in large scale monocultures is likely to continue this trend of new pests emerging over time.

## MAJOR PESTS & CURRENT RESEARCH

### Erinose mite

This mite was first detected in 1999/2000 near Somerset Dam and outbreaks have been observed each year in an increasing proportion of the plantation estate. The mite also occurs on spotted gums in northern NSW, but until recently had not been recorded causing significant damage. Growth can be severely affected, with losses of up to 55 per cent height growth and 35 per cent diameter growth recorded over 18 months depending on site and climatic factors. Loss of stem form is also potentially associated with infestations of the mite. Significant differences in susceptibility to the mite have been found between provenances of spotted gum and other *Corymbia* species. Some N-NSW CCV provenances, *C. torelliana*, *C. henryi* and the CCV x CT hybrid all show high levels of tolerance to the mite compared to Woondum CCV, which is highly susceptible. A large scale quarterly survey of the incidence and severity of mite damage has been carried out in all DPIF plantations over the past 12 months and the data is currently being spatially analysed for trends in the epidemiology of this mite. A preliminary survey of a smaller number of plantations in May 2004 showed that: (a) damage was widespread over all regions where spotted gum is planted in SEQ, (b) western and southern plantings had a higher proportion of severe and moderate damage than other regions, and (c) severe damage was recorded only in two- and three-year old plantings.

### Paropsis atomaria

This beetle has a very wide geographical distribution extending from central Queensland along the east coast to southern Victoria and west to South Australia, and broad host range of around 20 eucalypt species. From 1997 to 2002 it was mostly observed defoliating plantations of *E. cloeziana* (and to a lesser extent *E. pilularis*, *E. dunnii* and *E. grandis*), but in 2003 caused significant defoliation for the first time to a number of CCV plantations. It has also recently been recorded causing severe defoliation to *E. grandis* x *E. camaldulensis* hybrid eucalypt plantations in the Miriam Vale area. With its wide host range and distribution it seems to have the ability to switch to whatever is the most widely available resource in a particular area, and is therefore a threat to spotted gums and other *Corymbia* spp plantations. Current research on this beetle is focussing on (a) refining a population model to assist in defining risk and evaluating management methods and (b) investigating methods of enhancing natural control mechanisms in plantations.

## **Winter Bronzing Bug**

*Thaumastocoris* sp. bugs were first recorded damaging a spotted gum planting in the Burnett in May 2004. These bugs are capable of causing significant defoliation (foliage “bronzing”) to trees, and have been outbreaking on a number of eucalypts in the Sydney region in recent years, including on some *Corymbia* spp. Little is known about the biology of this insect or its host range. Plots were set up in August 2004 at the most severely affected site to monitor effects of the bug on growth and will be reassessed over the next month. Susceptibility of other *Corymbia* taxa is yet unknown.

## **Stem Borers**

CCV and CCC in trials and plantations established since 1997 have shown very good resistance to stem borers. *Corymbia* is generally not considered to be a host for the giant wood moth (*Endoxyla cinerea*) but can be attacked by longicorn beetles, especially if trees are highly stressed. In assessments of nine trial plantings over a wide range of sites mean incidence of attack on 2.5 to 3 y.o. CCV by the two-hole borer, *Phoracantha solida*, averaged only 0.6% with a maximum of 9.5% at a particularly harsh site. Trials of the *Corymbia* hybrids have yet to be intensively assessed for borer damage but anecdotal evidence suggests they may be more susceptible to borers than is CCV. Although not yet observed in plantations, another longicorn, *P. mastersi*, has been recorded doing extensive damage to *Corymbia* in native forests.

## **Red-shouldered leaf beetle (RLB) *Monolepta australis***

Although not recorded as a significant pest in plantations of CCV in SEQ, *C. torelliana* is known to be highly susceptible to damage by RLB and is often planted in orchards as a windbreak and trap tree for RLB. In a trial planting of CCV, CCC, *C. torelliana* and hybrids established at Walkamin in 2000, only *C. torelliana* was severely attacked by RLB – the hybrids were not attacked at all, even in plots adjacent to *C. torelliana*. However, it is possible that different crosses/families of the hybrid may differ in their susceptibility of this insect, so monitoring of trials and future operational plantings will be required.

## **Mistletoe**

These parasitic plants have been flagged as a potential health issue in plantations and Dr Angus Carnegie will address this pest in detail elsewhere in this workshop. In monitoring plots set up for bronzing bug in a four-year old plantation (see above) 11/80 trees (14%) had mistletoe associated with them.

# FORESTS NSW *CORYMBIA* BREEDING AND DEPLOYMENT PROGRAM



Helen Smith

Tree Improvement Program, Land Management and Technical Services, Forests NSW.

Forests NSW, a part of the NSW Department of Primary Industries, currently manages approximately 50 000 ha of hardwood plantations. *Corymbia* species comprise around 8 000 ha of the young (post-1994) plantations. In 1995 a breeding program was initiated to support the renewed operational eucalypt planting program. Problems that have limited the productivity of the plantations have included susceptibility to frost, Ramularia shoot blight caused by *Quambalaria pitereka* and more recently susceptibility to the erinose mite *Rhombacus* sp. Selection of superior individuals in the tree improvement program is aimed to address these problems, as well as select for good growth and form.

## PROGENY TRIALS

Phenotypic selection of plus trees in native forests throughout the range of *Corymbia citriodora* subsp. *variegata* (CCV) in NSW and south-east Queensland commenced in 1995. In March 1999, a progeny trial of CCV, containing 194 families, 113 from NSW and 81 from south-east Queensland, was established at a site near Bonalbo, north-west of Casino. Concurrently a planting was made on a property west of Casino, of seedlings of 17 families of Woondum provenance, with the intention of using it in the future as selection blocks and potentially a seed production stand.

In early 2001, two further progeny trials were established, at sites south of Grafton and west of Casino, to supplement the earlier breeding population. Each site contained 85 families; these trials also had 29 and 30 families in common with the 1999 trial. The trials were predominantly comprised of families from Woondum provenance, plus 22/23 families (from 12 NSW provenances) that had shown field tolerance to Ramularia leaf blight during assessment of the 1999 progeny trial.

During 2004, a further series of four progeny trials of CCV was established, each comprising of between 526 and 544 families. A total of 251 families were from 26 NSW provenances and 293 families were from 16 Queensland provenances. These have 104 families in common with the 1999 trial and 22 families in common with the 2001 trials.

This has resulted in Forests NSW breeding population of CCV comprising of approximately 687 families in pedigreed trials over seven sites in northern NSW. In addition, in early 2004 progeny trials of *Corymbia maculata*, containing up to 140 families, were established on three sites.

This season, it is planned to plant progeny trials containing the four main *Corymbia* species, CCV, *C. maculata*, *C. henryi* and *C. citriodora* subsp. *citriodora* on two sites. These trials will comprise of over 460 families, including some new collections of CCV from Richmond Range.

## CLONE TRIALS

Along with the planting of progeny trials, clone trials of CCV were established on multiple sites to allow selection of clones for commercial deployment and to study clone by site interaction. During 1999, four sites were planted to clone trials containing 69 clones from 9 selected plus trees families from 5 NSW provenances. Plants were propagated by

micropropagation from aseptically germinated seedlings. After assessment in 2001 and 2002, a subset of 33 clones was selected on growth, *Ramularia* incidence and Crown Damage Index (CDI) for further testing. In 2003, these clones were planted in a series of four trials, the plants again being propagated by micropropagation. Seven of the best performing clones were selected for further multiplication for commercial plantations.

## **COMMERCIAL DEPLOYMENT**

For the past two planting seasons, propagation of selected tested CCV clones has been underway to produce plants for operational plantings. In the first season, approximately 1.1 ha was planted to CCV clones, but in the past planting season this was increased to more than 10 000 plants (10 ha) being produced for operational plantations. A similar propagation and planting program is planned for next season.

Selected trees from the three earliest progeny trials have been grafted over the past two years and Forests NSW first clonal seed orchard has just been established. Selections were made on growth, form (straightness), *Ramularia* shoot blight tolerance and frost tolerance. Another clonal seed orchard will be planted next spring on a different site, to manage risk.

Future deployment of improved material will be via two sources. Selected tested clones will continue to be propagated for operational plantations. In addition, a program of Improved Family Forestry will be initiated using the improved seed produced from the clonal seed orchards. Techniques for screening very young seedlings for traits such as *Ramularia* shoot blight tolerance are being investigated. Propagation of plants is by micropropagation and rooted minicuttings and significant effort is currently being made to improve these techniques to make them more cost effective.

## **HYBRID CLONES**

Hybrids between *C. torelliana* and other *Corymbia* species have become of interest in recent years due to traits that *C. torelliana* are thought to impart in the other species. These include field resistance to *Ramularia* shoot blight and frost, faster growth rates that lead to more rapid capture of the site, reducing weed and pest problems, and good rooting of hybrids with *C. torelliana* as the mother. During 2005, Forests NSW has established two trials to examine the potential for these hybrids on NSW sites. One of these is in conjunction with DPI&F, Horticulture and Forestry Science Qld, testing some of their *Corymbia* hybrids.

## **DRYLAND TRIALS**

Forests NSW has been involved in the Australian Low Rainfall Tree Improvement Group (ALRTIG). Under the auspices of this project a progeny trial of CCV was established in the Upper Hunter Valley in October 2001, to test the performance of CCV in lower (600-700 mm) rainfall areas on a northern inland NSW site and to potentially be developed into a seedling seed orchard, producing seed for inland commercial plantings. The trial contained 120 families, 83 from 13 Queensland provenances and 37 from 5 NSW provenances. Unfortunately, severe drought and frost conditions during 2002 resulted in very poor survival and the trial being written off.

## CORYMBIA GENETICS AT SOUTHERN CROSS UNIVERSITY

Mervyn Shepherd<sup>1&2</sup>, David Lee<sup>3</sup>, Natalie Baker<sup>1&2</sup>, Shabana Kasam<sup>1</sup>, Joel Ochieng<sup>1</sup>,  
Chen Bihua<sup>1</sup> and Robert Henry<sup>1&2</sup>

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<sup>2</sup> CRC for Sustainable Production Forestry

<sup>3</sup> DPI&F Horticulture and Forestry Science

Research on *Corymbia* spp. genetics at Southern Cross University focuses on molecular tree improvement but also encompasses studies on population structure, comparative mapping and phylogenetics. The Politaria section (Spotted gums) of *Corymbia* contains four taxa including *C. maculata* and *C. citriodora* ssp *variegata* (CCV). The first of these is one of the top ten planted eucalypts worldwide, whilst CCV is a priority plantation species for saw logs in Queensland and NSW. Two projects are underway to support improvement programs for spotted gums and their hybrids. The first project will study the genetics of propagation characteristics in an intersectional *Corymbia* hybrid (*C. torelliana* x CCV, a taxon of emerging importance for forestry in subtropical regions of eastern Australia. The approach taken here is to develop models of genetic architecture from genetic mapping and quantitative trait loci (QTL) analysis in a segregating family. In a second project, association tests will be used to establish genotype-phenotype links between candidate genes for solid wood characters in “natural” populations of CCV. A project examining the population structure of the spotted gum complex is being undertaken to support association studies (this will be reported upon separately by Joel Ochieng).

Cytologically *Corymbia* may be distinguished from *Eucalyptus* by a more symmetrical karyotype and a genome with around ½ the DNA content. We are examining the extent of genome size variation within the *Corymbia*, *Angophora* sp. and hybrids to explore the taxonomic value of this character. We will also use comparative mapping to study whether structural rearrangements (translocations and inversions) or gross genome organisation (the grouping and ordering of genes within its genome) changes have accompanied the change in DNA content during the evolution of the *Corymbia* genome since its divergence with *Eucalyptus*.

Once considered a subgenus of *Eucalyptus*, the relationship of the *Corymbia* genus (bloodwoods and the “ghost gums”) within eucalypts in the broadest sense (*Angophora*, *Corymbia* and *Eucalyptus*) remains controversial. The existence of a dichotomy, the Angophoroid clade (*Angophora* and *Corymbia*) and a non-Angophoroid clade (*Eucalyptus* ss), is widely accepted; however the relationships within the Angophoroid clade remain much less clear, with incongruence between morphological and DNA sequence data. A key issue is whether the *Corymbia* genus is monophyletic. In collaboration with researchers from the University of Tasmania, we propose to extend the intergenic transcribed sequence (ITS) phylogeny within the Angophoroid clade and identify new loci to provide additional characters to increase the confidence of the molecular phylogeny.

### COMPARATIVE MAPPING

Orthogonal microsatellite loci are being identified as a foundation for comparative mapping. A set of around 105 polymorphic microsatellite loci have been identified for the development of a framework map in *Corymbia*. These loci were identified from either *Corymbia citriodora* subsp. *variegata* (n=28) or from either *Eucalyptus grandis* or *E. globulus* (n=272). Of these, around 90 are highly conserved and amplify in both *Corymbia* and *Eucalyptus* and will facilitate comparative analysis. A downside to transferring loci from *Eucalyptus* to *Corymbia*

was a reduction in polymorphism (PIC value reduced from 0.8 to 0.6 for transferred loci) but if a locus transferred, it had a low rate of single-allele nulls in a CT x CCV mapping cross.

### **GENETIC MAPPING OF VEGETATIVE PROPAGATION CHARACTERS IN A *C. TORELLIANA* X *C. CITRIODORA* SUBSP. *VARIEGATA* F<sub>2</sub> HYBRID**

Segregation analysis is being conducted in an F<sub>2</sub> population (n=208) generated from parents divergent in propagation characteristics. Genotyping of around 25 microsatellite loci has been completed. Nursery trials to evaluate shoot production, rooting percentages and root quality of stem cuttings are also underway. Seedling germination and rooting of stem cuttings was low in this second generation hybrid compared to F<sub>1</sub>, perhaps indicating outbreeding depression. However, while the family average was low (~15% for rooting percentage), variation within the family was extensive and clonal repeatabilities were moderate and highly significant (r=0.48 p-value <0.01). Shoot production also appears to be under strong genetic control (r=0.75; p-value < 0.01).

### **MOLECULAR PHYLOGENY**

We have collected material from an additional 36 species from *Corymbia* and 2 *Angophora* to extend the ITS phylogeny. Sequencing has been completed for 5 species to date but we are encountering difficulties due to fungal contamination and universal ITS primers. We are currently attempting to re-design more specific primers to complete this work.

### **MOLECULAR VARIATION IN CCR IN THE SPOTTED GUMS: A PRECURSOR FOR ASSOCIATION STUDIES**

Cinnamoyl CoA Reductase (CCR) is involved in lignin biosynthesis and has been associated with cell wall stiffness and strength properties in *Arabodopsis* and *Eucalyptus globulus* and therefore is a leading candidate gene for control of solid wood characters in spotted gums. We are currently characterising variation in this 3.2 kb gene in spotted gums.

### **ACKNOWLEDGEMENTS**

This research is funded by the ARC, DPI-Forestry and the CRC for Sustainable Production Forestry with in-kind support from the Centre for Plant Conservation Genetics SCU and DPI&F Horticulture and Forestry Science. We also thank the following people and organisation for assistance with material and advise; John Oostenbrink, Peter Pomery and Garth Nikles (DPI/F) Rhonda Stokoe (USC), Dean Nicolle (Currency Creek Arboretum), Micheal Henson (Forests NSW), David Kleinig (Dendros Seed), Rachel King (Griffith Uni), Dot Steane, Rene Vaillancourt and Brad Potts (CRC SPF and U of Tas), ATSC, Lana Little (QNP&W), Tim Willing (CALM), Don Rielly (DBIRD), John Woinarski (DIPE) and Kings Park Botanical Gardens.

## POPULATION STRUCTURE IN THE SPOTTED GUM

Joel W. Ochieng (PhD candidate)

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The major objective of my project is to determine the extent and causes of population structure in the spotted gums complex. Knowledge of population structure is important for valid interpretation of association tests, an approach increasingly adopted for molecular tree improvement. In this project, I will focus on testing for two common causes of structuring in plant populations, geographic isolation and hybridisation.

My study will focus on *Corymbia variegata*, a taxon of key commercial importance for hardwood plantations in Queensland and New South Wales. *Corymbia variegata*, like most eucalypts (and trees in general) that are predominately outcrossing and widely distributed, is expected to have most variation within populations and little amongst populations, and therefore weak structuring as a consequence of isolation by distance. Perhaps of greater consequence for structuring, is the potential for hybridisation between closely related taxa that occur in sympatry. Although *C. henryi* and *C. variegata*, are recognised as separate species, there is growing evidence that they may be simply morphotypes of a single species. The implications for population structuring differ depending on whether the taxa behave as one or more gene pools.

In this presentation I will report on a pilot study testing for structuring based on taxon boundaries. I found there was no evidence for structuring *C. henryi* and *C. variegata* along taxon lines based on five nuclear microsatellite loci. No unique alleles or significant allele frequency differences were found to distinguish the two taxa; consequently there was low genetic differentiation amongst geographically paired individuals from the two taxa ( $F_{st}=0.005$ ). This suggested there was high level of gene flow between the two taxa. A low but significant structuring was evident within each taxon (*C. henryi* overall  $F_{st}=0.01$ ; *C. variegata* overall  $F_{st}=0.02$ ), and fitted an isolation-by-distance model, indicating geographic, rather than reproductive barriers, was more important for structuring in these taxa. Phylogenetic analysis based on allele sharing statistics showed a similar trend as the  $F_{st}$  analysis, with geographically proximal provenances of *C. henryi* and *C. variegata* showing the highest affinity. Outlying provenances, not previously included in population studies of these taxa (Ewingar, Mt. Barney, Nerang, and Lockyer), were amongst the most differentiated, as expected from their geographic remoteness. Mt. Barney in particular was the most separated population. There was also a clear north-south division aligning with a population disjunction that corresponds to the east-west running NSW-Queensland border range. Only one out of the five markers (*EMCRC37*) showed positive results for pair wise linkage disequilibrium tests across all populations, suggesting that this locus could be under selection. The implications of population structuring and linkage disequilibrium for association studies are discussed.

## **BREEDING SPOTTED GUMS FOR WINTER-DOMINANT RAINFALL REGIONS OF AUSTRALIA: AN OVERVIEW**

Warburton, P.M., Arnold, R.J., Brammall, B.L., Harwood, C.E., Larmour, J.S., McDonald, M., Owen J.V, Whitfeld, S.J. and Bush, D.J.

ensis Genetics, PO Box E4008 Kingston ACT 2604

### **INTRODUCTION**

CSIRO FFP (now ensis) first became involved in spotted gum tree breeding in the early 1990s. Since that time there have been changes in *Eucalyptus* and then *Corymbia* taxonomy, heightened interest in low rainfall plantation forestry and a marked expansion of the spotted gum estate in the northern NSW and southern QLD summer-dominant rainfall region. Advances have been made in the state of knowledge on flowering, vegetative propagation, frost tolerance and species/provenance selection. Seed orchards have been established to make available genetically improved germplasm. The focus of this presentation is on spotted gums for plantations on sites receiving winter-dominant rainfall in temperate, southern Australia.

### **TARGET SITE TYPES IN THE WINTER RAINFALL ZONE**

The perceived target planting zone for the spotted gums in south-eastern Australia includes both higher rainfall sites east of the divide, and medium-low rainfall sites in the sheep-wheat belt west of the divide. Spotted gums are considered to be key 'commercial environmental forestry' species for the latter region, where land is likely to be more readily available. Despite the predominantly coastal distribution of *C. maculata* (CM), the species has been shown to perform well on more favourable sites in the sheep-wheat belt. 'Favourable' sites are those with reasonably deep, fertile soils that are free from waterlogging; higher rainfall, not below 600 mm MAR, and/or some access to ground water and; few or no heavy frosts and and/or a position in the landscape with good cold air drainage.

In Western Australia, CM has also been shown to perform quite well at the climatically moderate western fringe of the southern sheep-wheat belt. However spotted gums tend to suffer from form problems caused by parrot damage to leading shoots. It is unknown to what extent this will limit the usefulness of the spotted gums there.

### **SPECIES: INFORMATION FROM SPECIES-PROVENANCE-FAMILY TRIALS**

The species recommended for plantation forestry in southern Australia is CM (Mazanec and Harwood 2001). This is because in the main, CM provenances tend to be more vigorous than *C. citriodora* ssp. *variegata* (CCV) and *C. henryi* (CH) on winter rainfall sites, where *Ramularia* does not impact on growth and survival. However, there are important exceptions to this general rule, with some families of CCV and CH typically amongst the top-performers in southern Australian family trials. Integration of such non-maculata families into southern spotted gum breeding populations is therefore worth considering, though not necessarily straightforward to achieve.

### **MOLECULAR GENETIC INFORMATION: TAXONOMY AND GENETIC RELATIONSHIPS WITHIN THE SPOTTED GUMS**

A study of the spotted gums was undertaken using allozymes markers to elucidate the genetic relationships within the section *Politaria*, and to inform breeding programs (McDonald *et al.* 2000). Key findings include:

- a high level of genetic diversity within a narrow geographic range of *C henryi*

- relatively low genetic diversity of other spotted gums
- high differentiation among populations of *C. citriodora* and *CM* (barriers to gene flow)
- support for the intergrade between *C. variegata* and *C. maculata* reported by Hill and Johnson (1995)
- southern populations of *C. citriodora* more akin to *C. variegata* than the northern populations
- *C. variegata* not distinct from *C. citriodora* (two chemotypes) suggesting former should be considered a subspecies of the latter (taxonomy now revised to *C. citriodora* ssp. *citriodora* (CCC) and *C. citriodora* ssp. *variegata* respectively – McDonald and Bean 2000).

This information has had significant implications for the management of existing mixed species seed orchards, and in the creation of tree breeding strategies for the spotted gums.

## **DOMESTICATION AND TREE BREEDING**

A number of organisations have carried out domestication and tree breeding activities with spotted gums in southern Australia. These include Forest Products Commission of Western Australia (FPC), Victoria's Department of Primary Industries, and ensis-CSIRO with collaborators. Together with Forests New South Wales (which also has a summer-rainfall-focused spotted gum program – see pages 19-20 in this publication) and ForestrySA, these organisations, have developed a collaborative breeding program under the Australian Low Rainfall Tree Improvement Group (ALRTIG). This program is based on a breeding population of *CM* which is managed as a series of progeny trials, some of which will be thinned later to produce seed. ensis also manages, in partnership with others, mixed species spotted gum seed orchards and a pure *CM* grafted *CSO*. FPC also has a spotted gum grafted *CSO* that is now producing seed which has superseded their earlier seed production areas.

Traits of interest in the first generation of ALRTIG and ensis programs have included stem straightness, branching, forking and vigour. In later generations, frost tolerance and wood properties might be included, if demand for the species justifies the breeding program investment.

A series of genetic gain trials was established by ALRTIG with industry partners in 2003. These trials compare *SSO CM* with 'seed stand' *CM* and *CCV*, plus wild *CM* and *CCV*. These trials will provide important information on the value of the breeding programs, and allow stakeholders to test the material using their preferred silvicultural treatments and on their target site types.

## **ORCHARD MANAGEMENT ISSUES**

Current ensis seed production capacity is mainly from two mixed-species spotted gum orchards. The orchards, situated at Holbrook and Deniliquin NSW, were created by selectively thinning species/provenance/progeny trials containing *CM*, *CH* and *CCV*. The orchards contain 9 provenances/76 families and 16 provenances/100 families respectively. These *SSOs* have provided good opportunities to compare the species' flowering phenology. The three species peak at different times (usually *CH* first, followed by *CCV* in summer, then *CM* later in autumn-winter), but some overlap has been observed between all three in some years. Bud initiation to flowering takes ~24 months at these inland orchard sites, and though flowering is often heavy, retention of buds to the harvestable capsule stage is poor, estimated at circa 20%.

Demand for seed has mostly been for *CM*, and crops have been collected from the best individuals. Mean multi-locus outcrossing rates in the *CM* have been acceptable ( $t_m=0.76$ ) at

the Deniliquin seed orchard, as determined by isozyme studies (McDonald 2004), though individual trees ranged from 0.5 to 0.9. The low outcrossing rate in some trees may be due to the combined effects of non-synchronous flowering and restricted pollen flow caused by localised buffering by trees of different species in the multi-species orchard. Single species designs of the type established by ALRTIG are probably preferable in spotted gum open pollinated orchards.

## **FROST TOLERANCE**

Lack of frost tolerance (particularly during the first year or two post establishment) is a serious limiting factor to plantation establishment in southern Australia. Laboratory cold exposure studies were done on families of CCV, CCC, CM and CH (Larmour et al. 2000). Measurement of electrical conductivity of cold-exposed leaf leachates showed that, generally, CCV was significantly more cold resistant than the other species. Also, inland, higher altitude provenances of CCV and CH were significantly more cold resistant than coastal material. There may therefore be potential for integrating frost tolerance into later generations of the southern spotted gum breeding populations. However, before this can be done, the growth performance of the more cold resistant material must be assessed (these provenances were often not included in early trials) and the correlation between laboratory-determined cold resistance and field-based frost tolerance needs to be gauged. FNSW have also made selections from the survivors of ALRTIG's only CCV trial, which was largely frost-killed in its second year post planting. This material is also of interest for further frost studies, and potentially, breeding.

## **VEGETATIVE PROPAGATION**

Recent studies (Brammall and Harwood 2001 and ensis unpublished data) on improving the rooting of temperate dry-zone species, including CCV and CM, show that hydroponic mini-cutting systems can significantly improve rooting over conventional stem cuttings for some species, though for CM the average rooting percentage was only around 19% using macro cuttings and 25% using mini-cuttings. CCV rooting success using mini-cuttings was around 45%. In both CM and CCV in the mini-cutting study, the existence of hard- and easy-to-root families was made clear across a number of experiments involving various hormone treatments and hedge plant harvests.

## **FUTURE DIRECTIONS**

Future priorities might include the development and assessment of interspecific hybrids on both winter rainfall and drier, less *Ramularia*-prone areas in the summer rainfall belt. Also, integration of cold tolerant material into existing southern breeding populations would improve the environmental plasticity of the genetically improved material. While it has been established that spotted gum wood has characteristics that lend it to high strength sawn applications (Blakemore et al. 2002), little has been done to determine whether important heritable wood property traits (e.g. tension wood, colour, natural durability) exist. Such traits might also be integrated into later generations. Development of a protocol for vegetative propagation would have obvious benefits, and work already carried out suggests that this should be further investigated. The intensity of R&D undertaken on these activities will depend largely on demand for the species for southern sites and drier summer rainfall sites, though much of the research would also have important implications for the summer rainfall spotted gum programs. Exchange of information and possibly genetic material between the programs is likely to be of mutual benefit.

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# CLONAL VARIATION IN SUSCEPTIBILITY OF *CORYMBIA CITRIODORA* SUBSP. *VARIEGATA* IN SUSCEPTIBILITY TO RAMULARIA SHOOT BLIGHT AND ERINOSE MITE IN NEW SOUTH WALES

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## BACKGROUND

Forests NSW established four *Corymbia citriodora* subsp. *variegata* clonal trials in early 2003, with 33 clones and a selection of families. These 33 clones were selected from earlier, larger trials, based on growth, form and Ramularia shoot blight (RSB) tolerance. The four trials are situated near Bonalbo, north of Grafton, and north and south of Coffs Harbour, in north-eastern NSW. Early growth, form and RSB were measured at ~12 months. In April 2005 all trees in the four trials were assessed for damage from RSB and Erinose mites (*Rhombacus* sp.), using the Crown Damage Index (= crown severity).

## RESULTS & DISCUSSION

### *Erinose mites*

We observed wide variation in susceptibility to Erinose mite, with a single clone being significantly more damaged than all other clones or families. At one site (Emu Creek), this clone sustained over 50% CDI, whereas all other clones were less than 15% CDI. At Zuills, this clone sustained 15% CDI compared to 1% for most other clones.

The Emu Creek trial (near Bonalbo) sustained significantly more damage to all clones (both incidence and CDI) than the other 3 sites, with the two coastal sites (near Coffs Harbour) with very few trees that had recordable levels of Erinose mites. Emu Creek is inland, and hotter, and less humid than the other sites, with the coastal sites having higher humidity and more rainfall.

In forest health surveys of *Corymbia* spp. plantations in the past 10 years, we have seen little damage from erinose mites, with low levels of incidence and severity observed. They were first observed in 1997, and have been recorded in plantations from Bulladelah north. We have also observed *Rhombacus* sp. causing damage to *Angophora costata* around Sydney.

### *Ramularia shoot blight*

There was also significant variation in damage from RSB; however, this was variable, even at the clonal level. The vast majority of trees in all trials had no damage from RSB, with a few individuals with significant damage and shoot death. This indicates that under the climatic conditions of the last 1.5 years the vast majority of clones showed very high tolerance to RSB infection.

Recent surveys of 8–10-year-old plantations, that had sustained high levels of RSB during their first few years, revealed that only a low proportion of trees (1–5%) still had significantly high levels of RSB and shoot death.

# MISTLETOES IN YOUNG *CORYMBIA* SPP. PLANTATIONS IN NORTHERN NEW SOUTH WALES

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## INTRODUCTION

Parasitic mistletoes are a common sight in native stands of eucalypts in Australia. Several mistletoes, including species of *Amyema* and *Muellerina*, have become significant problems in remnant stands of eucalypts in rural areas, being associated with significant growth reductions (up to 50%) and mortality of mature trees Reid & Yan, 2000<sup>6</sup>). Although common in native forests in north-eastern New South Wales, mistletoes have not reached levels to warrant concern or remedial action in commercial forests. Moreover, mistletoes have not previously been reported as a problem in eucalypt plantations in Australia.

Due to an increase in the presence of mistletoes being observed in young spotted gum plantations during regular forest health surveys, we made a concerted effort over the past few years to assess the majority of 5–10-year-old plantations for the incidence of mistletoes.

## RESULTS AND DISCUSSION

We observed significant levels of mistletoe (*Amyema* spp. and *Dendrophthoe vitellina*) in young (5–10-year-old) eucalypt plantations in north-eastern NSW. Of the four main species of eucalypts planted in NSW, *Corymbia citriodora* subsp. *variegata* is the most susceptible to mistletoe infestation. Plantations rarely started to become infested with mistletoe below 5 years old. Of ~75 plantations over 5 years old, 20 had mistletoe, ranging from 0.1% to 68% incidence. Ten plantations had less than 5% incidence, 7 stands had 5% to 25% incidence, one had 40% incidence and two over 65% incidence. The incidence of mistletoe in these 5–10-year-old plantations is expected to increase over the life of the rotation, and the proportion of plantations with significant levels of mistletoe is also expected to increase.

The impact of mistletoe in young eucalypt plantations, and possible management strategies, is currently being examined by NSW DPI & Forests NSW.

Significant levels of mistletoe in young *Corymbia* spp. plantations have also been observed in south-east Queensland (S. Lawson, pers. comm.).

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<sup>6</sup> Reid, N. & Yan, Z. (2000). Mistletoes and phanerogams parasitic on eucalypts. *In* Keane et al Diseases & Pathogens of Eucalypts. CSIRO.

# INCREASING PLANTING SUCCESS OF *CORYMBIA VARIEGATA*

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## INTRODUCTION

Successful establishment of forestry plantations relies on many factors including high survival of transplanted seedlings. Survival of seedlings is influenced by many factors including seedling vigour, location within the landscape, availability of moisture at time of planting, and weather conditions after planting. In this series of experiments we examined the use of water retention gels to increase the seedling available moisture, leaf coatings to reduce transpiration water loss, and altered nursery practices to produce hardier seedlings more able to withstand the temporary 'drought' conditions associated with planting seedlings.

## INITIAL FIELD TRIALS - USE OF GEL AND INFLUENCE OF SOIL MOISTURE AND WEATHER

Water retention gels offer an alternative risk management strategy under poor planting conditions. Their use also offers the opportunity to greatly expand the planting window, especially in the most desirable planting season in spring when soil moisture may be the only limiting factor. Four sites were selected to cover a range of north to south, coastal to inland and different soil types. Soil moisture on the day of planting were marginal at the time of planting. At each site seedlings were planted using one of two treatments, namely with a gel or as per normal practice. In gel treated plots a small hole was dug and 1 litre of hydrated Alcosorb® was mixed with soil and the seedlings planted. Seedlings were planted as per normal practice with a pottiputki in all control plots.

The sites had a range of responses from almost 100% survival in both control and gel treated plots to greater than 90% mortality in the treated plots and very low survival in the control plots. The gel did not improve survival on only one occasion and gave excellent survival under all but the most extreme planting conditions. This suggests that gels have the potential to greatly increase the planting window, and if used routinely under marginal conditions would greatly reduce the need to infill. A complex interaction between moisture availability and weather conditions after planting on seedling survival is apparent. This interaction is being explored in order to develop a decision support system to determine the optimum planting window.

## FINE GRADE GEL AND KAOLIN CLAY LEAF COATING

Increasing moisture availability and reducing seedling transpiration water loss are two methods by which seedling survival may be enhanced. Earlier research showed hydrated gels increase the availability of water to seedlings and promotes higher seedling survival. However, the application method can be costly as mixing the hydrated gel into the soil at the time of planting was the only method that enhanced seedling survival when coarser grained gels were used. Finer grained gels form a smooth mixture that adequately coats the root plug of seedlings when they are dipped into a hydrated solution. These coated seedlings can be planted using conventional means such as Pottiputki's which greatly reduces cost compared to using the coarser gels.

Reducing seedling transpiration may decrease seedlings deaths associated with dehydration but could promote high leaf temperatures and therefore seedling death. The Surround WP®

particle film, a kaolin based product, transmits photosynthetically active radiation, while its reflective properties has been shown to reduce heat build-up and heat stress in leaf canopies of mature crops (Glenn et. al. 2001, 2003). These properties ultimately increase the amount of assimilate available for growth and reduce water use and increase water use efficiency (Glenn et. al. 2001, 2003; Jifon and Syverston, 2003). We coated leaves of seedlings with Surround WP® prior to planting to determine if the coating promoted seedling survival.

Our field experiment was located on a sandy loam near Casino. Moisture at planting was low ( $0.08\text{g g}^{-1}$ ) in the sandier top soil but considerably higher ( $0.16\text{g g}^{-1}$ ) in the heavier subsoil. Survival was confounded by over 20 mm rain in the first week and regular follow-up rain in the next 2 months which probably accounted for survival of 87 and 72 % in control seedlings of *C. variegata* and *E. pilularis* respectively. Survival of *E. pilularis*, but not *C. variegata* seedlings which had root-balls dipped in the gel was enhanced over non-coated seedlings. A follow-up study in a glasshouse where no irrigation was provided showed survival of seedlings of both species was enhanced by dipping the root-ball in the gel. Coating the leaves with a Kaolin clay compound did not affect survival. This finding did not appear to be related to rain washing the coating off the leaves as a trial in a glasshouse also showed no effect of the leaf coating on enhancing seedling survival. Use of the finely graded gel will reduce the risks associated with planting in less than optimal conditions.

## **HARDENING SEEDLINGS TO DROUGHT PRIOR TO TRANSPLANTING**

Plants may be acclimated or hardened to water limiting conditions. This hardening can occur in the nursery by reducing the frequency or amount of irrigation provided to the growing seedlings. The drought hardening procedure has been shown to assist in survival and or growth after transplanting of many crops including radiata pine, cypress pine, apricot, tomato and lotus (Rook, 1973; Ojemakinde and Onwueme, 1980; Arnott et al., 1993; Ruiz-Sanchez et al., 1998; Franco et al. 2001). These adaptations are probably related to increased root : leaf area ratio which would enhance water absorption and reduce seedling water loss; as well as changes in more fundamental mechanisms related to limiting leaf transpiration or enhancing root growth.

The hardening procedure involved progressively reducing the amount and frequency of irrigation provided to the seedlings in the month prior to transplanting. Reducing irrigation initially increased wilting of the seedlings but this effect was short lived. The hardening treatments did not increase seedling mortality while the seedlings remained in the nursery. The field experiment was run in conjunction with the field experiment described above (Dipping root ball in gel and Kaolin clay leaf coating). Hardening seedlings to drought resulted in fewer seedlings deaths following transplanting than unhardened seedlings. This effect did not appear to be related to an increased root : leaf area ratio as artificially removing leaves (top-clipping) did not enhance survival. Hardening seedlings to drought while they remain in the nursery is a low cost management tool which will increase the economic success of plantation forestry as costs of re-planting and infill of poorly established plantations will be reduced.

# SOIL PHYSICAL LIMITATIONS TO GROWTH IN SUB-TROPICAL HARDWOOD PLANTATIONS

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## BACKGROUND

This project aims to correlate the productivity of eucalypt plantations with site data to develop a model to predict growth rates on new sites. The model derived as part of this project will allow accurate prediction of growth rates and therefore economic viability of plantation in defined areas. The geographical applicability of the model, limited by the range of the sites examined, will be North-Eastern NSW but the methods developed will have much wider usage.

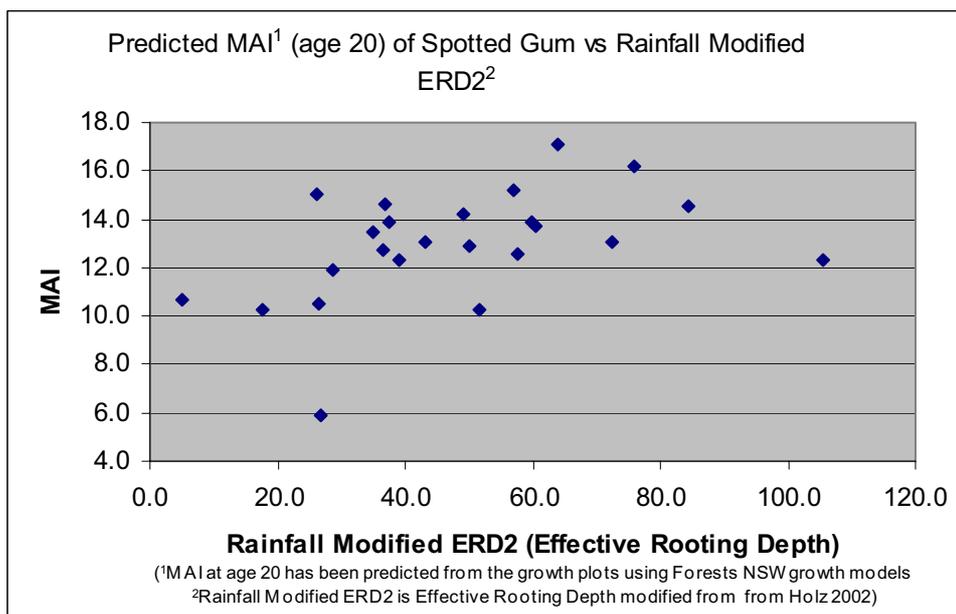
In order to aid in the understanding of soil characteristics on root growth and plantation productivity part of the project aims to examine the alternative root growth strategies that two important hardwood plantation species (Dunn's white gum and spotted gum) use to exploit soils with a range of different physical properties.

Spotted gum is considered specifically for this paper.

## RESULTS

### *Site Productivity Prediction*

Site and soil assessment and description have been carried out across a set of permanent growth inventory plots established by Forests NSW in their hardwood plantations. This data along with the growth data being provided by Forests NSW is being combined with actual and predicted climatic data for the plots to create the model. An initial comparison of site and soil characteristics using a site selection model developed by SF NSW and growth indicates promising correlations exist (see diagram below), particularly as some of the outliers can be identified as being due to specific site limitations not otherwise considered.



Preliminary analysis suggests that the main growth determining factors for spotted gum at these sites are soil structure, depth and drainage rather than nutrients. The next steps to be taken in analysis will include analysis of climate information, full nutrient data and site and soil characteristics and should lead to a more reliable site productivity estimate system.

*Plantation Root Growth as Determined by Soil Characteristics*

The examination of root growth is in progress with three sites selected (two Brown Dermosol soils and one Yellow Chromosol). Pot trials on undisturbed, intact soils taken from these sites have been established with spotted gum, Dunn's white gum, silky oak and blackbutt.

**CONCLUSIONS**

Results have not been examined in enough detail at this stage to draw any definite conclusions. However, preliminary results highlight the importance of detailed assessment of soil physical characteristics in site productivity prediction. Nutrient levels appear to have less potential in predicting productivity potential on a wide scale.

## **QUAMBALARIA PITEREKA ON SPOTTED GUM PLANTATIONS IN QUEENSLAND AND NORTHERN NEW SOUTH WALES, AUSTRALIA.**

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The establishment of a sustainable hardwood plantation industry in Subtropical regions of Australia will require effective pest and disease control strategies to be developed. Spotted gum (*Corymbia citriodora*) is a valuable commercial timber and suitable for a wide range of different soil types in large parts of Queensland and New South Wales. The main biological constraint to further expansion of spotted gum plantations is the disease Ramularia shoot blight caused by the fungus *Quambalaria pitereka*. Ramularia shoot blight, caused by the fungus *Quambalaria pitereka*, has been found in most spotted gum plantations in Queensland and northern New South Wales. *Q. pitereka* infection often results in the repeated destruction of the growing tips resulting in the formation of a bushier crown or death of the tree in severe cases. Spotted gum growth rates can also be negatively impacted upon. Any impact on the growth rates in the first 2 years after planting will impact negatively on the formation of the butt log, the most valuable part of the log. Forest health surveys indicate that disease incidence can reach 100% with an average of 25% of the trees showing moderate to severe infection levels at the time of inspection. Initial studies into the population of *Q. pitereka* have indicated variability at the morphological and genetic level. A degree of variation in virulence between isolates has also been shown.

The project aims to provide essential information for future tree breeding programs, silvicultural programs and species selection for plantation managers in both private and public forestry enterprises. The research will provide information on the biology of the pathogen and the influence of environmental factors, which will assist in the development of disease management strategies. The host pathogen interactions will provide information on host aspects associated with disease tolerance and/or resistance. Investigations into the efficacy of chemical control programs will assist in developing a control strategy for established plantations.

1. Determine the biology of *Q. pitereka* to enable the development of effective disease management strategies.

### **Outcome**

- Determine the source of inoculum.
- Determine if *Q. pitereka* is an endophyte.
- Determine if *Q. pitereka* colonises inflorescences.
- Determine mode of infection.
- Initiate research into the influence of environmental factors on *Q. pitereka*.

2. Determine if *Q. pitereka* is a highly variable fungal pathogen and provide information on host susceptibility for future tree breeding and plantation species selection.

### **Outcome**

- Collect and culture *Q. pitereka* isolates from Queensland and northern New South Wales for population analysis.

- Determine differences between *Q. pitereka* and *Q. eucalypti* from South Africa and investigate management strategies adopted to control *Q. eucalypti* in South Africa.
  - Initial investigation into determining if there is a specific host pathogen interaction.
3. Determine the effectiveness of plant defence promoters in reducing the impact of *Q. pitereka* on spotted gum plantations.

**Outcome**

- Initiate glasshouse trials to determine the influence of a range of products with defence promotion qualities.
- Initial results on concentrations of chemical additives to be used to reduce disease severity.