

## Breeding of radiata pine in Australia

McRae<sup>1</sup> T A, Buxton<sup>1</sup> P A, Pilbeam<sup>1</sup> D J, Dutkowski<sup>2</sup> G W, Kerr<sup>2</sup> R J, Ivković<sup>3</sup> M and Wu H X<sup>3</sup>.

<sup>1</sup>Southern Tree Breeding Association Inc. PO Box 1811, Mount Gambier, SA 5290, Australia

<sup>2</sup>PlantPlan Genetics, PO Box 1811, Mount Gambier, SA 5290, Australia

<sup>3</sup>CSIRO Plant Industry, Canberra, ACT 2601, Australia

Email: [tmcrae@stba.com.au](mailto:tmcrae@stba.com.au)

**Keywords:** tree breeding, genetic improvement, radiata pine

The objective of the national tree improvement program for *Pinus radiata* in Australia is to breed, select and deploy genetic material with improved biological characteristics for traits of commercial importance. Breeding began in the 1950s with plus tree selection and the establishment of seed orchards by various companies and state and federal agencies. The STBA breeding cooperative, established in 1983 in South Australia, continues to expand, consolidating genetic resources on a national basis, providing cost efficiencies and enhancing the rate of genetic gain for this major plantation species.

Economic objectives for plantation growing for structural timber markets are fundamental to the breeding and deployment programs. Changes in the environment due to changing climate and markets are evaluated within this framework.

There is effectively a single breeding population with a single national objective. We are still deciding on how best operationally to manage the population for regional performance, different products and risk traits associated with some pests and diseases. New software tools (SELECT and MATE) have helped breeders manage relatedness and limit the change in group co-ancestry to ensure inbreeding does not build up too quickly in the advanced generation population. Some effort is also directed towards conserving native provenance material for risk management purposes as well as infusion of genes from tested selections to broaden the genetic base.

The breeding program moved to a rolling front strategy in 2000, where breeding, testing and selection activities are done on an annual basis. This has reduced costs and generation interval by avoiding resource and biological bottlenecks, and maintained the skills of field technicians and breeders (particularly in reproductive biology). It has enhanced the rate of genetic gain per unit time, as well as delivering new information and genetic material (tangibles) to cooperative members on a regular basis. Infusion of 30-50 new selections each year into a dedicated multi-age breeding arboretum allows about 250 crosses pa - many more than was previously achieved per generation.

Progeny tests and deployment trials are established on an annual basis, and across years to sample all of the plantation estate. Families are also being exchanged with the RPBC in New Zealand for collaborative testing in series of trials across Australasia. Families with parents in common provide genetic linkage among trials across sites and years. Treatments are stratified into groups of families with similar breeding values for growth to reduce competition among single tree plots and provide large plots to monitor

long term stand growth. Traits measured at various ages include growth, tree form, wood properties (density and stiffness using acoustic tools), and damage due to pests (pine aphid) and diseases (dothistroma, pine pitch canker, phytophthora and spring needle cast).

All data and information is stored in the national DATAPLAN database. The web based system has been operating since 2001 and currently stores ten million measurements on 650,000 *Pinus radiata* genotypes in over 300 trials, with a total of 60 million measurements on four million genotypes in 11 species. We are currently incorporating new trial data (more than 80 trials) for radiata pine from a previously independent state based breeding program in WA, following Forest Products Commission joining the national cooperative in 2013. This will further broaden the genetic base and increase selection intensity for all members.

The TREEPLAN genetic evaluation system has been used routinely since 2001 for breeding value prediction using multi-generation multivariate (-site, -trait and -age) BLUP. BLUP allows us to account for previous selection and monitor trends in genetic improvement over time by having objective comparisons across time and space. Variance components are estimated within trials (and across subsets of trials) using ASREML and other tools. TREEPLAN genetic values (EBVs, EGVs and SCAs) are currently predicted for 32 selection criteria (measured traits) using data from more than 160 trials in the multi-variate analysis. Genetic values are generated for the clearfall harvest age breeding objective traits of growth (MAI on a regional basis, m<sup>3</sup>/ha/yr), stem straightness (SWEEP, mm/m), BRANCH size (cm), and timber STIFFNESS (GPa). Economic indices using appropriate economic weights (based on various production systems and end use processing) are produced for each genotype, family, aggregate and genetic group.

Standard errors and accuracies are now routinely reported as measures of reliability for all types of genetic values for all pedigree entities (individuals, families, aggregates and genetic groups), and for all trait types (selection criteria, objective traits and profit indices). Genetic values for all the population are updated regularly (at least annually) as new data is collected. Realised gain trials are used to audit the predictions and ensure scaling factors are appropriate, particularly for MAI. Implementation of changes in economic models and other assumptions derived from research projects is straightforward within this framework.

The STBA does not itself produce seed and plants for deployment. This is done by members and a licensee. STBA provides selections for use in propagation systems, as well as objective information to assist seed producers, nurserymen, and forest growers make informed decisions about what material is best for their situation. This is not a simple task, as the breeding program generates complex and vast amounts of information for consideration in deployment.

The SEEDPLAN decision support system has been developed within the DATAPLAN and TREEPLAN framework for industry use after pilot testing. Its purpose is to improve the use of improved seed and clones in deployment under different situations. Tools assist with selecting genotypes to best balance gain and diversity (SELECT), placement of ramets in seed orchards (DESIGN), estimating the composition of seed harvested (COMPOSE), and customising the economic indices for the different growing

environments and production systems (INDEX). A tool for allocating seed and plant lots to stand types in an optimal manner to maximise enterprise profitability (MATCH) has been prototyped, but is still under development.

Other studies have focused on juvenile wood and timber stiffness. The potential impact of climate change is given some consideration, but mainly in terms of characterising the genetic resource for a greater range of environments. A better understanding of GxE patterns and associated environmental factors is helping classify site types across Australasia for breeding and deployment. Current research suggests rainfall, temperature and altitude are largely responsible for observed patterns of genotype by environment interactions. It is anticipated some refinement of site type classifications for screening and deployment of genetic material will improve overall gain.

Molecular markers have been used in the past for fingerprinting to check pedigree error rates in the breeding and deployment populations. Genomic selection is not being used operationally in the tree improvement program for radiata pine at this time. Research projects have generated some marker data, but it has been too little to warrant incorporation in routine evaluation. It is anticipated next generation genetic values will increasingly rely on additional information from molecular genetic studies.

Breeding values incorporating non-neutral marker information can currently be done in TREEPLAN by using a synthetic pseudo-continuous trait. Multi-locus marker-trait responses are combined into a prediction of genetic merit for a trait based on genotypes which have phenotypic and/or marker information. This approach has been used successfully in *Eucalyptus nitens*. We anticipate advances in genotyping technology will make it feasible to test individuals for hundreds of thousands of single nucleotide polymorphisms simultaneously at an affordable price. A genomic relationship matrix based on neutral markers can be merged with the average co-ancestry matrix currently used. This should increase the accuracy of breeding value prediction, by better accounting for the Mendelian sampling term.

Alleles of large effect can be incorporated directly into the model, utilising all phenotypic data to better identify their effects. The opportunity for radiata pine is largely with harvest age traits that are only expressed later in the life of the tree, or are expressed rarely, such as with pests and diseases, or are very expensive or impractical to measure. It will be necessary to continue collecting phenotypic data to ensure precision of estimates.

In summary, the radiata pine program has undergone substantial change in the past decade with the development and adoption of economically defined breeding objectives, screening for new pest (essigella pine aphid) and disease traits (pine pitch canker), increased measurement of stiffness, development of DATAPLAN software and data consolidation in national database(s), development of TREEPLAN software for comprehensive industry wide genetic evaluation with regionalised genetic values, a rolling front operational program, development of selection, breeding and deployment (SEEDPLAN) tools, gene conservation coordinated nationally, improved security of the national genetic resource (breeding population), and a focused research portfolio with rapid adoption of results and findings.

## Breeding of radiata pine in Australia

TA McRae, PA Buxton, DJ Pilbeam,  
 GW Dutkowski, RJ Kerr, M Ivković and HX Wu

Southern Tree Breeding Association Inc.  
 Australia



## BACKGROUND

Plantation industry in Australia

Breeding began in 1950s

1983 STBA Inc.

Consolidation of private, state and federal programs into  
 a national program

Cooperative model provides cost efficiencies, better  
 security of resources, and more genetic gain

STBA just breeds (not seed since 2001)

STBA is involved in breeding multiple species



## Components of a breeding (tree improvement) program

- Economic breeding objective(s)
- Genetic resource
- Genetic architecture of the population(s)
- Breeding program
- Testing program
- Data recording and information management
- Data analysis and genetic evaluation
- Selection (for breeding and deployment)
- Deployment systems (companies)

Balance is needed

## ECONOMIC BREEDING OBJECTIVES

$$NPV\$ = w_1 \text{GROWTH} + w_2 \text{BRANCH} + w_3 \text{SWEEP} + w_4 \text{STIFFNESS}$$

Economic weights differ for growers and processors

We breed for an integrated enterprise

Flexible for each company



## Breeding and deployment values (NPV\$)



**Genetic resource  
 and understand the genetic architecture**

Origins of STBA program (multiple)

Genetic material (physical) and knowledge  
 (pedigree and performance data)

Consolidated across time and space

Plus infusions of genetic material from native  
 provenances and other programs (Australia, New  
 Zealand, Chile, Spain, Kenya, France and South  
 Africa)

**BREEDING PROGRAM**

Population improvement

Purchased land for dedicated breeding arboretum  
 (Mount Gambier 2005)

National objective is to breed for an integrated industry

But have other objectives (mainly for risk traits)

Rolling front strategy



Target 250 breeding crosses pa (~1400 last 10 yrs)

2013 season: 348 breeding genotypes (Mount Gambier)

237 flowering (census)

+ utilise deployment orchards for some crossing



**SELECTION  
 of genotypes for breeding**

We use the SELECT tool (runs from SEEDPLAN)

Maximise gain for the economic objective

Rolling front breeding scheme – the parental group is constantly  
 changing each year

30-50 new selections are grafted annually

The breeder must maximise genetic gain while considering the  
 build up of coancestry in a constantly changing group of  
 individuals, over a very long time horizon

Recruitment population (potentially all genotypes)

Use the RECRUIT tool (to reduce run time)



Now use a rifle



**Mate allocation in crossing**

We use the MATE tool

creates a crossing program based on maximising genetic  
 gain subject to constraints

Differential Evolution (DE) genetic algorithm to solve  
 the mate allocation problem

subject to constraints:

target effective population size of 400 (ensure slow build up of  
 coancestry that eventually translates to inbreeding)

minimise short term inbreeding in progeny resulting from the  
 mating plan

Considers pool of potential parents currently reproductively  
 available (census)



### TESTING PROGRAM

Different types of trials

Multiple sites (*different environments and manage risk*)

Progeny tests: 4 sites pa (25,000 trees pa)  
 250+ families  
 single tree plots (20 full sibs per site)  
 trial designs (row-column)

Embedded large plots stratified on growth EBVs  
 (calibration and reduce competition)

Ensure genetic linkage across sites and years  
 (50 F x 20 sibs per site)

Directors proofing (demonstration) trials as needed



### Collaborative progeny tests (2013 and 2014)

Collaborative tests across Australasia (STBA with RPBC)

Reciprocal exchange of families (75 F pa)

Target 4 sites (AUS and NZ) pa

Help benchmark populations

Provide genetic linkage across sites and years for future analyses

Enhances genetic diversity

Should enhance gain



### Assessment and measurement

Data for many traits

Growth and form

Wood quality more important in late 1990s  
 (basic density, Mfa, spiral grain, acoustic stiffness)

Pests (Essigella pine aphid) and diseases (Dothistroma, Phytophthora, Spring needle cast)

Risk traits (pine pitch canker and drought)

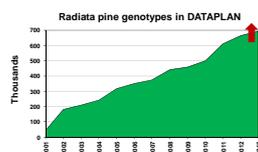
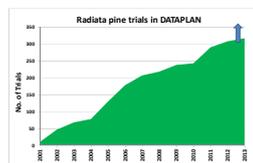
Traits are measured at different ages and not all trees in all trials are assessed for all traits (unbalanced)

Anticipate SNP marker data in future!

### Data and Information Management

We use DATAPLAN  
 Began transferring in 2001  
 Web based

Statistics:  
 321 trials (+ 85 FPC)  
 Many historical and research trials?  
 693,085 genotypes (of 3.6 M)  
 9,950,203 measurements (62 M)  
 9391 families (4662 CP)  
 18 regular users  
 Performance uptime 99%+



### Data analysis and genetic evaluation

We use TREEPLAN (since 2001)

Breeding and genetic values

Multi-generation multivariate BLUP (-site, -trait and -age)

Aim to use all data and full pedigree (species wide evaluation)

Variance components (within and across subsets of trials) estimated using ASReml and other tools

We can monitor trends over time



### TREEPLAN evaluation

**What are the outputs for radiata pine?**

**Genetic values (EBVs, EGVs and SCAs) for 32 SC traits**

growth 7 regions (3 age classes)  
 form (BRA, BRQ, BRS, HF deformity, stem straightness)  
 quality (density 2 age classes, spiral grain and MOE)  
 diseases (SNC, Phytophthora, Pitch canker, Dothistroma)  
 pests (pine aphid)

**Genetic values for BOT traits**

regionalised growth (MAI m<sup>3</sup>/ha/yr)  
 BRANCH size (cm)  
 stem straightness (SWEEP mm/m)  
 timber STIFFNESS (GPa)

**Genetic values for all genotypes, families, aggregates and genetic groups**

**Economic indices for all ..**

**Accuracies and SE's for all genetic values and indices**




### TREEPLAN evaluation

**An example**

**Statistics: PRAD2013**

**Date: 10 July 2013 (run time 1:55)**

**Trials: 161**

**Genetic groups: 27**

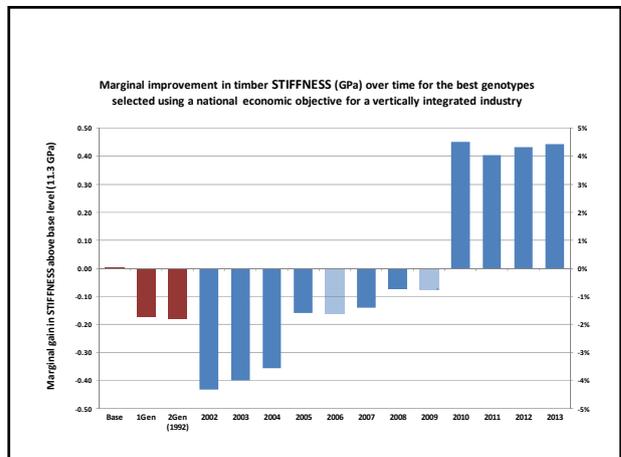
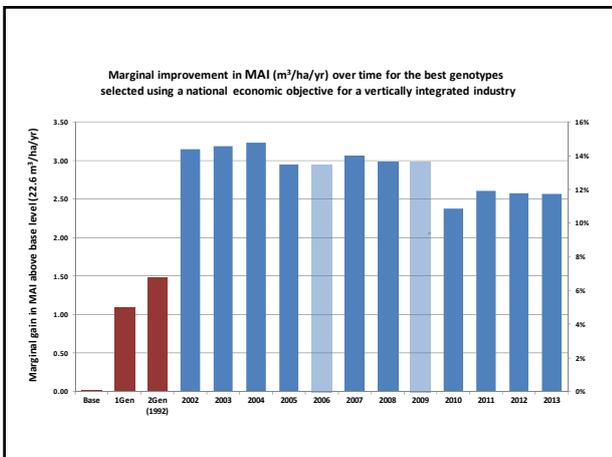
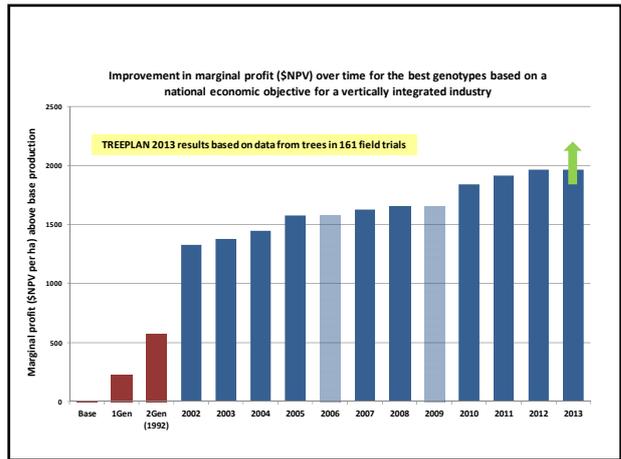
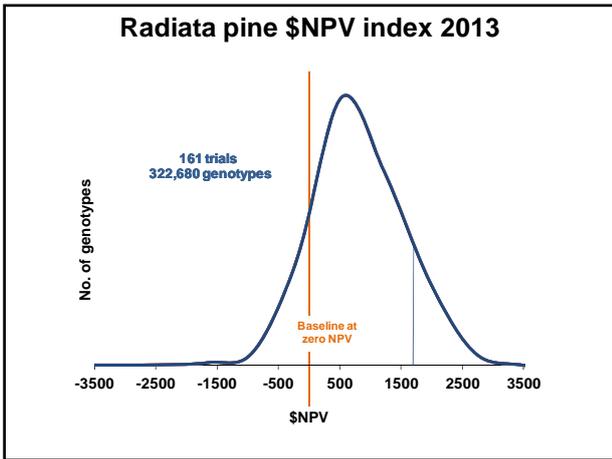
**Families: 8093 (2 AxA, 135 GxG, 413 lxA, 2993 lxG, 4534 lxl, 16 MxM)**

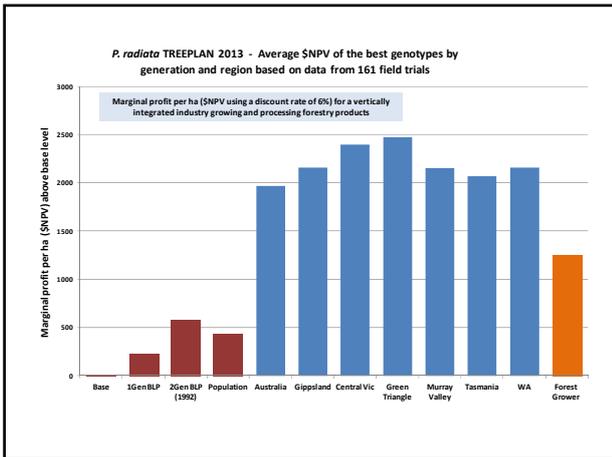
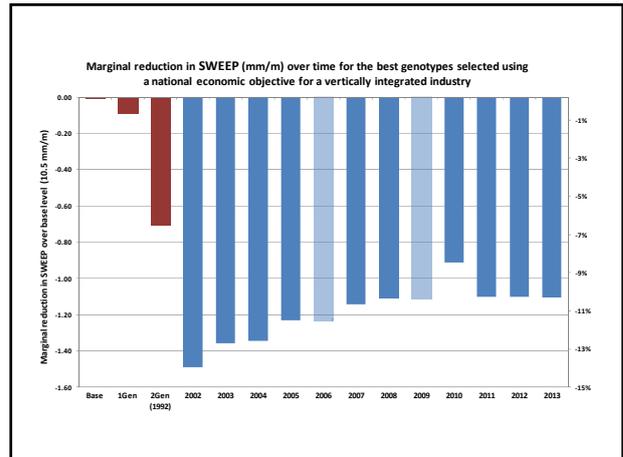
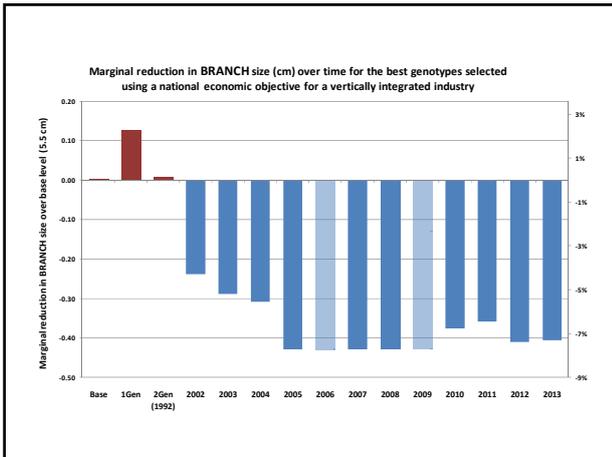
**Genotypes: 322,680**

**SC traits: 32**

**BOT traits: 12**

**Baseline: 219 genotypes (1G selections)**





## SEEDPLAN II project



Optimal use of genetics in deployment and tree breeding  
 STBA group (and partners) project with FWPA (funding)

We have all this genetic and economic information, but how do we use it to match genetics to different site types and production systems.

Deployment is complex  
 Project is a lot about technology transfer



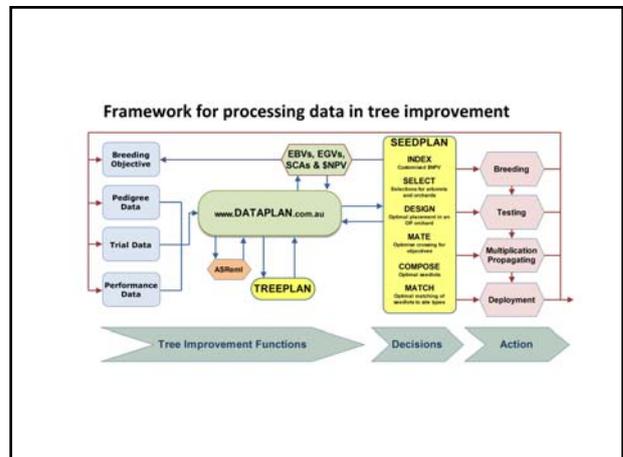
## SEEDPLAN tools

SEEDPLAN is a collection of tools that aids our breeders and deployers to carry out common tasks

- SELECT** (selecting genotypes)
- MATE** (mate allocation)
- DESIGN** (orchard design)
- COMPOSE** (seed lot values)
- INDEX** (customise the index)
- MATCH** (matching to sites)

**Other tools** (Pedigree extractor, RECRUIT, STATUS, CO-ANCESTRY)

Read the **SEEDPLAN USER GUIDE**

## GENE CONSERVATION

Introductions of provenances (Monterey, Año Nuevo, Cambria Gudalaupe and Cedros Islands) in the past

FWPA project

Unable to import radiata pine into Australia

It can be fashionable



## Studies on GxE

Milos Ivkovic (CSIRO) will discuss details

GxE is important for growth

TREEPLAN breeding values for growth traits are regionalised

But, this only partly accounts for GxE

We need to better explain patterns and environmental drivers

E (scale) impacts on genetic values and economic weights

We are somewhat limited by useful data in Australasia

Genetic linkage across sites and years (and programs) is paramount for future studies of GxE



## RESEARCH PROGRAM

STBA facilitates (resources and \$)

Research associates do the research

Recent topics:

economic objectives, juvenile wood initiative, GxE, genetic diversity, genomics, inbreeding, Essigella pine aphid, pine pitch canker etc

Systems and tools provide a platform for incorporating results and findings into the commercial program



## Genomics and molecular breeding

Progress: fingerprinting (was helpful)

largely platform building to date

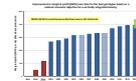
no impact on genetic gain for economic objective in radiata pine

What about the future?

handle major gene effects directly

synthetic pseudo continuous trait for multi-locus markers

merge GRM with NRM to increase accuracy of prediction and account for Mendelian sampling



## Changes since 2000

Changed to a rolling front strategy (rather than cycles)

Adoption of economic objectives (structural industry)

More focus on wood properties

Some new pest and disease challenges (threats)

Better security of genetic resources (breeding and conservation)



National database established (DATAPLAN)

Multivariate BLUP is done routinely (TREEPLAN)

Developed some SEEDPLAN tools for breeding and deployment tasks

A more focused research portfolio with a clearer pathway to adoption



### **Future developments**

- Need to capitalise on the investment in genomics**
- Monitor breeding objectives (environments and products)**
- Help mitigate climate change (moving targets are a challenge)?**
- Share data and information across programs to increase genetic gain and reduce risk**
- Define site types better to account for E and GxE**
- Be more efficient with less resources (its expected)**

### **Acknowledgements**

