Tree Breeding and Genetic Deployment

Presentation to the Farm Forestry Course, July 1997

Dr Liz Barbour, Dept of CALM

***Richard Mazanec** has kindly offered to present this lecture.*

INTRODUCTION

At the recent Farm Forestry Annual conference David Boomsma (Southern Tree Breeders Association) presented a paper on "Prospects for future plantations - tree breeding" which gave a good overview of the various breeding programmes that have been established in Australia in the last fifty years. To save repeating this information, I have decided to take a specific tree crop as an example of tree breeding. The one that seems to generate the most interest is the *Eucalyptus globulus* breeding programme which we have named the "Western Blue Gum". It is a good example of a major area of R&D currently being undertaken by CALM.

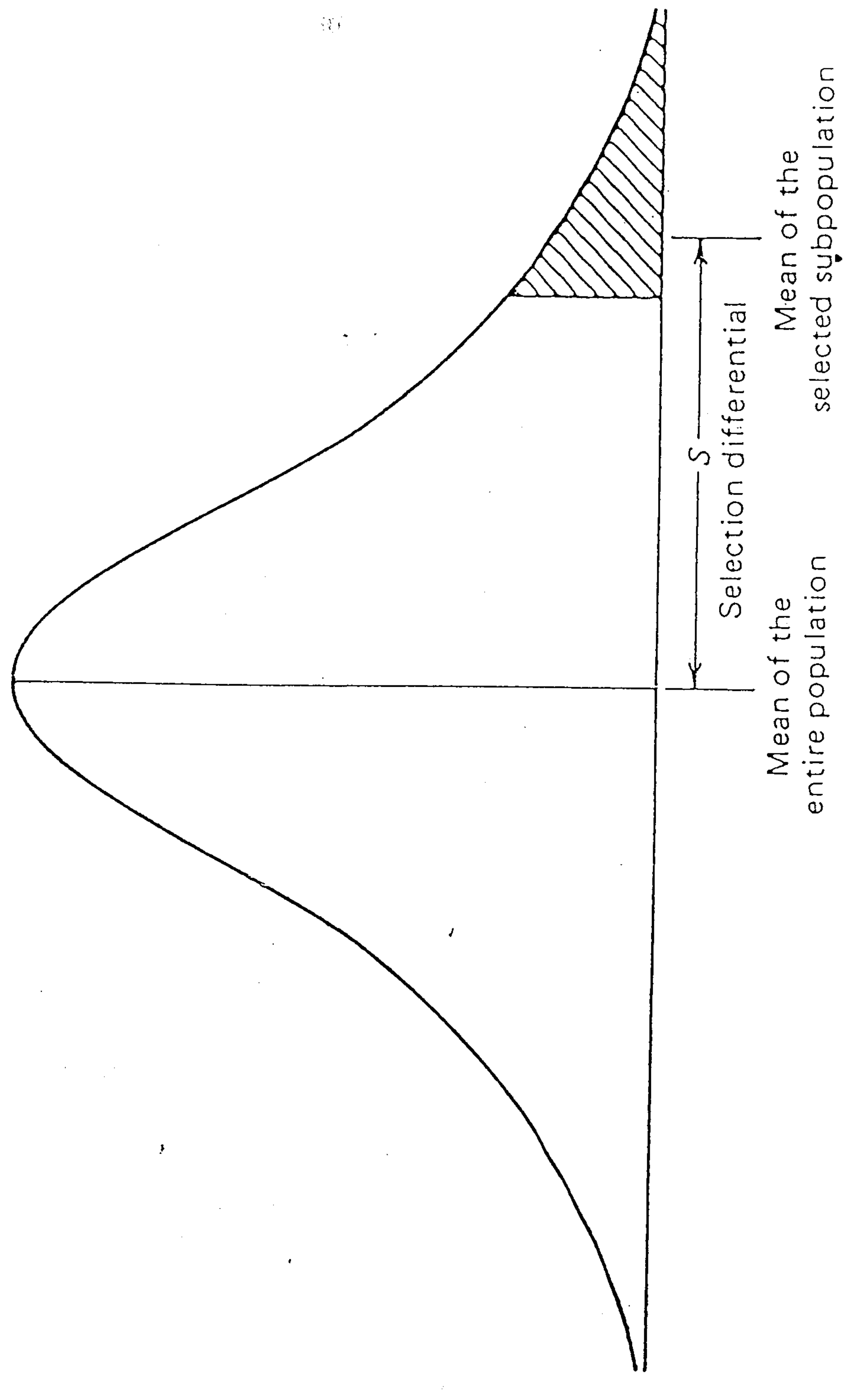
The easiest part of this breeding programme was the marketing of the product. The "Western Blue Gum" name and logo indicates that the seedlings have been produced from seed from seed orchards based on the CALM *E.globulus* breeding programme and are therefore genetically improved. This talk was originally only to cover tree breeding but from experience it has been found that one of the hardest tasks is to pass on breeding gains to the grower. Usually a breeder will identify single trees or families of trees that have the superior genes. The challenge remains to multiply these few superior selections into the millions required for plantation establishment. This is what we term "Genetic Deployment". Whilst the breeding of *E.globulus* has been relatively easy (good seed collections, shorter rotation time, shorter maturation time for selection) compared to the pines, the deployment of these gains has been a fascinating challenge. So we will give you a broad introduction to breeding and add an insight to the choices for genetic deployment.

TREE BREEDING

> *"In a forest plantation a genetically poor tree will continue to produce below the potential of the land and the climate resource for as long as it occupies the site ..."*
>
> *"Investment in genetically good trees will maintain above average growth on the land resource and will continue without further cost ..."*
>
> *(Libby et al., 1969)*

This is Trevor Butcher's (our senior Tree Breeder) favourite quote. Tree breeding is about increasing the profit from a tree. It ties the land rehabilitation role of the tree to an industrial product which creates the income. A tree breeding programme is designed to increase the efficiency of the species to produce a chosen product.
This graph (*Figure 1)* represents how gains are made in tree breeding.

Figure 1: Quantitative aspects of forest tree improvement.



There are two basic requirements:

1. A population must be established that represents the native population (which is the full curve of the graph). This is termed "Base population".
2. Secondly methods must be used to be able to identify and select the top portion of the range (the shaded area) to improve the population for the next generation.

These statements are over-simplified as you will begin to appreciate as we go further into understanding tree breeding.

1. Base population

1.1 Taxonomy

To create a base population, you first need to understand the taxonomy of the species you are dealing with. *E.globulus* is an interesting species. It belongs to the Myrtaceae and has been divided into 4 sub-species by Kirkpatrick in 1974 (*Figure 2).* The four sub-species are identified by their bud configuration:

- *E.globulus spp globulus* -large single budded inflorescence and largest fruits
- *E.globulus spp pseudoglobulus* - 3 budded smaller fruits and longer pedicels
- *E.globulus spp bicostata* - 3 budded fruits, slightly larger and sessile pedicels
- *E.globulus spp maidenii* - buds largely in 7's rather than 3 or single.

1.2 Distribution

E.globulus is native to Eastern Australia. (*Figure 3).* The species is divided into its four sub-species and then further divided into provenances. This diagram shows the first provenance collection made of *E.globulus* by Keith Orme.

1.3 Seed collecting

There are certain guidelines that need to be followed during the seed collecting process to capture a good representation of the native population.

The seed collector must be trusted to:

- *locate all the possible populations of the species*
- *correctly identify the species and collect a herbarium sample*
- *collect seed from one parent in a group of trees with a 100 m distance between the trees (Figure 4)*
- *seed must not be collected from isolated individuals*
- *individuals with good healthy growth and form should be selected*
- *code the seed collection and record detailed field data including the tree position and a description of surrounding environment.*

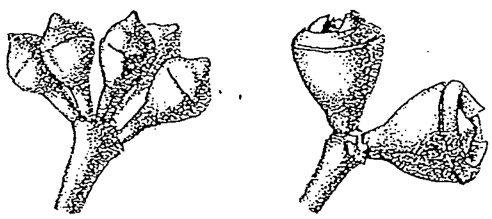
Our Western Blue Gum base population has been established from a number of seed collections. The first was done by Keith Orme and the CSIRO Australian Seed Centre was sponsored to do more extensive collections. This same thing happens here for

Figure 2: Buds of the four <u>E.globulus</u> sub-species showing the bud configuration to differentiate between the sub-species

E.globulus spp globulus

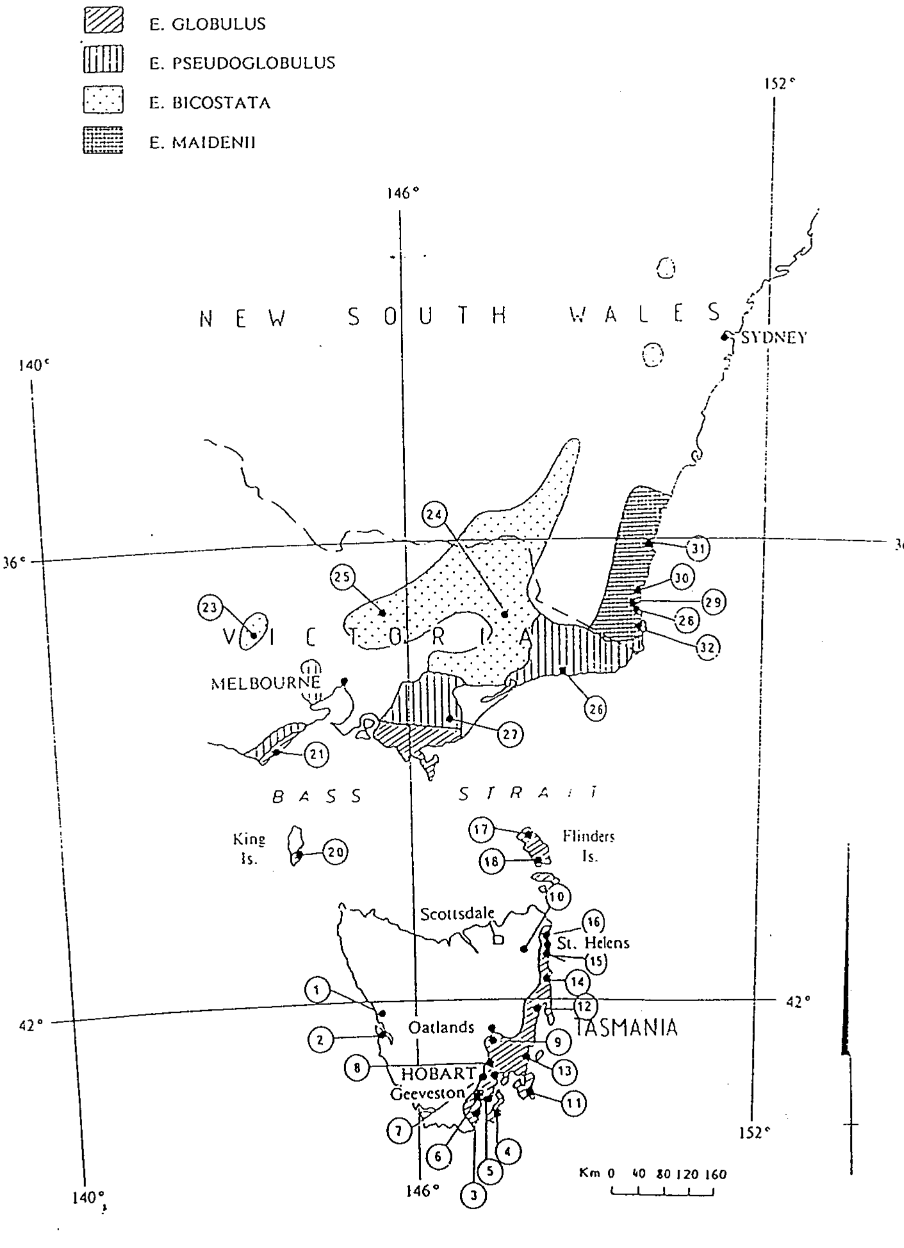
E.globulus spp psuedoglobulus

E.globulus spp bicostata



E.globulus spp maidenii

Figure 3: The native distribution of E.globulus



Western Australian native species; external companies sponsor the CALM Seed Centre (Manjimup) to do provenance and family collections. Perhaps the success of the Eucalypt world-wide can be attributed to the success of the Australian Seed Centre. Good breeding collections are often difficult to buy.

1.4 Sub species, provenance and family level trials

The first set of trials tested all four sub-species. Our Western Blue Gum population includes the subspecies globulus and psuedoglobulus.

A formal definition of provenance:
the original geographical area from which seed or other propagules were obtained (Callaham, 1964).

Tree breeding trials normally test the provenances with each provenance being represented by a number of families to capture the range of the provenance.

A "family" is the seedlings raised from the seed collected from a single tree

A good indicator of the depth of a breeding programme would be the number of families represented in the base population and more importantly, the number of individuals each family is represented by. For the Western Blue Gum base population CALM has planted over 1000 families with each family represented by at least 100 individuals. This makes this base population the largest for this species in the world. Thus when a top family is identified, there are enough replications to make the selection statistically sound without further testing.

2. Trial design

All trial designs should include the three basic rules of experimental design: replicate, randomise and have a control.

There are other guidelines that are taken into account when a trial is designed:

- *never use less than four replications for any test, six are preferable*
- *establish tests on each major soil type where operational planting is being considered*
- *the trial should be surrounded by a buffer zone of trees of the same species to avoid an edge effect. Normally the buffer zone or guard rows are two trees wide*
- *the trial should be silviculturally managed in the same manner as the situation into which future trees will be planted eg. land preparation, fertilising, spacing , thinning etc.*
- *assess these tests for survival and growth: at an early age, at expected half rotation and full rotation. These regular measurements will give the breeder the information by which early growth predictions of mature growth can be expected.*

3. Breeding terms

3.1 Traits

There are various attributes or "traits" that make a tree profitable for a specific product.

For the WBG we have concentrated on a number of traits that make economic sense for a wood pulp product:

- wood volume and wood density (which are the main contributing factors to bone dry units which is how the wood chips are presently sold)
- drought-tolerance and salinity tolerance (to extend the available land range)
- Pulp yield and quality (which may be important in future years for marketing our product)

3.2 Heritability

Forest trees are among the most variable of all living organisms; trees have a high level of heterozygosity. This large amount of variation is beneficial to the tree breeder but it tends to obscure differences. Although some of this variability is environmentally caused, a great deal has a genetic basis. Heritability indicates the proportion of the trait that is genetical. Thus the greater the heritability, the greater reward a breeder will have for selecting the trait. To know where the greatest rewards for breeding will be, a breeder has to set up trials to calculate each traits heritability.

The gain from a selection program is determined by the heritability (the strength of inheritance) and the selection differential (the amount of variation between the select and base populations)

$$G = h^2 \, X \, SD$$

G = gain, h^2 *= heritability* *SD = selection differential*

Some characteristics are more heritable than others, so by calculating the heritability of different characteristics from your trials will give you an indication of the success you will have selecting for that characteristic.

Carolyn Raymond did an excellent review of the Eucalypt literature on calculated heritabilities and found that all of the wood and fibre traits appear to be under moderate to strong genetic control (Raymond, 1995)

Table 1: Summary of the individual heritability estimates for a range of wood and fibre traits (no. is the number of estimates)

Trait	No.	Range	Mean
Basic density	16	0.05 - 0.84	0.57
Kraft pulp yield (%)	4	0.30 - 0.56	0.43
Soda demand	3	0.20 - 0.74	0.50
Moisture content	4	0.28 - 0.82	0.48
Fibre length	4	0.12 - 0.59	0.39
Pilodyne penetration	2	0.21 - 0.78	0.49
Fibre diameter	1	0.89	0.89
Lumen diameter	1	0.59	0.59
Vessel diameter	1	0.67	0.67

E. polybractea. oil yield. 0.45—0.59 0.53.

3.3 Breeding trait correlations

In the measurement and analysis of these trials it is important to understand the relationship between different traits of a species. It is easier to breed for different traits if they are not closely related genetically, or have a strong correlation.

For example, if stem straightness and growth rate are correlated, it would be difficult to select for a fast growing tree that was straight. The lack of correlation of adaptive (against disease, insects, adverse sites or weather) verses form and growth characteristics is important for the potential to develop profitable well-adapted strains of trees.

3.4 Genotype x Environment Interaction (GEI)

Sometimes trees will respond differently relative to each other in different environments. When this involves an actual change in ranking this is the most important type of interaction. When the change involves an altering of productivity from one environment to another with no change in rank then it has no effect on breeding. This GEI can change with the trait being studied.

The only way to assess GEI is to test more than one environment.

4. Summary of the breeding and genetic deployment process

Figure 6: Summary of the breeding and genetic deployment process

BREEDING	GENETIC DEPLOYMENT
<u>**Native seed collection**</u> ▼	
Sub-species level ➡	***Seed production areas*** *(random mix of seed)*
Provenance level	
<u>**Family level**</u> ➡ ▼	**Open Pollinated Seedling Seed Orchard F1** (OPSSO)
Best families identified ➡	**Backward Open Pollinated Seedling Seed Orchard F1** (BOPSSO)
Best individuals identified ➡	**Clonal Seed Orchard F1** (CSO) and Rooted cutting programme for clonal forestry
sub-lining of population breeding population. Crossing between best individuals	
<u>**F2 generation**</u> ➡ ▼	OPSSO F2
Best F2 families identified ➡	BOPSSO F2
Best F2 individuals identified ➡	CSO F2 and Rooted cutting programme for clonal forestry
Crossing between best F2 individuals	
<u>**F3 generation**</u> ➡	OPSSO F3

5. Breeding strategies

The plan of how a species is bred is written into a breeding strategy. This is an active document which can change as information is gathered. It is like a business plan. It will document:

- understanding the species and the <u>*breeding system*</u>: natural out-crossing, selfing, ability to control pollinate and vegetative propagate
- the <u>*base population*</u> - where the native seed collections were made and listing the trials and the material included in each trial

- how the selections were made for the *breeding population* and what they are. It has been calculated that a robust breeding programme needs in the range of 300 selections in a breeding population to maintain genetic diversity.
- *assessment* methods of the base population for each trait
- It will go on to discuss how the breeding population was *sub-lined* and this will be determined by the method of genetic deployment. Each sub-line population will be documented and will be kept separate throughout the breeding programme. One approach to sub-lining is if the genetic deployment through seed orchards is favoured, the breeding population will be divided into 5 sub-lines to ensure out-crossing in an orchard. If the genetic deployment is to be through vegetative propagation means, then the breeding population will be sub-lined into two. All crossing for breeding gains will occur within the sub-lines. Additional gains are made in the deployment population when there is out-crossing between the sub-lines.
- The *generation of the F2* is documented and this will include the crossing programme within each sub-line. In CALM all our breeding programmes have created the F2 using control pollination methods, which although expensive has many advantages. This means that both the male and the female pedigrees are known and this is termed *full sib*. Many programmes have favoured a *half-sib* approach which means that only the female is known. Two main advantages of a full sib F2 breeding population are that smaller sized trials are needed for the next generation and perhaps even more fortuitous for CALM is that full sib crosses open the doors to the biotech methods of DNA marker-aided breeding.

Whereas you look at profit to see if the business plan is working, for a breeding strategy, you look at the *genetic gain or yield trials*. These trials are established to test the predicted gains from a breeding programme. By using the top families identified in the breeding programme it was predicted that a gain of between 15-20 % could be made. The yield trials are young but they are showing 32% volume improvement to date.

GENETIC DEPLOYMENT

This is a subject within itself. Whereas Tree breeding is involved with statistical analysis, genetic deployment involves understanding the physiology of the tree and the development of propagation systems to multiply the selections. The breeding system of the species determines the opportunities

E.globulus has offered the greatest challenge. Not only is it one of the hardest eucalypts to stimulate adventitious rooting from but it also is a shy flowerer in Western Australia. As a result we have tried to maintain research programmes in as many propagation systems as we can hoping that one will work in the end.

The propagation systems we are researching are : seed orchards, rooted cuttings and tissue culture.

1. Sexual reproduction deployment through seed orchards

The different designs of orchard are developed with varying controls over the types of pollen available to each selection. The higher the genetic gain, the greater the control over the pollen available. This must be balanced with maximising out-crossing. Thus if all the trees flowered at the same time, it has been calculated that as low as 20 selections could be used to make an orchard. The advantage of using a small number of selections is that the better portion of the population can be used to make this orchard.
For example, the first clonal Western Blue Gum orchard was made with a broad choice of selections. If all the predicted volume gains of all the selections are averaged, the orchards volume gain is expected to be 40%. However there are some selections that are greater than 40%, some even reaching 80% volume improvement. Ideally the orchard should have been made of only these top selections increasing the predicted volume gain but until the flowering patterns are understood these gains can not be attained. Perhaps we are going ahead of our selves. Firstly we need to understand the different types of seed orchard that can be planted.

CALM has planted three types of *E.globulus* seed orchard:

- Open pollinated seedling seed orchards (OPSSO). The seed orchard is planted at the same time as breeding trials and as information is gained from the breeding trials the orchard is culled of the poorer trees. This orchard is at family level
- Backward open pollinated seedling seed orchards (BOPSSO). This seed orchard is only planted once breeding information is known so only the best trees are planted. We have planted a series taking into account growth data and are now planting a series based on flowering data. This orchard is at family level.
- Clonal seed orchards (CSO). This orchard is made up of grafts. Scion (branch material from a selected tree) is grafted onto a rootstock. In this manner only the very best trees from the breeding programme are incorporated into the orchard. This orchard is at the clonal level.

Many forestry companies have chosen seedling seed orchards (OPSSO and BOPSSO) over clonal orchards because of the expense and problems associated with producing the grafts. The gains for the Western Blue Gum population double (20% volume gain for BOPSSO compared with 40% for CSO) with using grafts and this we feel justifies the commitment to a clonal orchard programme. Additionally where we were initially having a poor grafting success (20%), research has improved this success rate to 80% dramatically decreasing the unit graft production cost.

The challenge with making eucalypts work in seed orchards is their flowering. Not only do the selections flower at different ages but within the flowering year, the flowering can span up to 8 months. Thus some selections do not naturally out-cross with others. As a result, in our orchard designs it has been important for us to understand the flowering pattern of as well as all the breeding traits.

2. Vegetative propagation - towards clonal forestry

Vegetative propagation can make identical copies of a selection as there is no unknown contribution of a pollen in the propagation system. As a result plantations can be made of the top selections in a shorter period than is possible by seed production from orchards. However, like seed orchards and the shy flowering, *E.globulus* is one of the poorest species of eucalypts in its ability to produce adventitious roots. Numerous research projects have been initiated to try and overcome this phenomenon environmentally and even with biotechnology (including infecting the base of the stem with root-promoting bacteria, *Agrobacterium rhizogenes)* but to date the only successful means of developing a vegetative propagation programme has been to screen for rooting. The ability to produce adventitious roots has been shown to be a heritable characteristic in *E.nitens* and it appears this may be true of *E.globulus* from the success of the screening programmes in Portugal. Thus if the unit cost of the screening procedure can be reduced, there may be an opportunity for genetic deployment for *E.globulus* through vegetative propagation.

Three methods of vegetative propagation of *E.globulus* have been investigated by CALM. Somatic embryogenesis which is the production of clonal embryos in tissue culture, micropropagation which is the multiplication and rooting of shoot tissue *in vitro* and rooted cuttings.

2.1 Somatic embryogenesis

The somatic embryogenesis project was halted after a year when little progress was made here or else where in the world. It appears this technique has been successful in other genera by catching an immature embryo development stage and causing it to multiply. This anatomical state appears not to occur in eucalypts and it will therefore require a novel approach to utilise this technique.

2.2 Micropropagation

The micropropagation project continues, not for production, but is being tested as a system to produce the mother plants for the rooted cutting system. It was found that the most efficient means of multiplication in this system was if the cultures were initiated from seed. As a result the system now multiplies the expensive control pollinated seed. This system is also being used to initially multiply the *E.globulus* hybrid material.

2.3 Rooted cuttings

The rooted cutting project is steadily increasing with the screening of good rooting clones which are being field tested each year. The rooting of *E.globulus* is generally very poor. Overall in our first screening after a year the rooting percentage was 19.18%. Some of the individuals gave 100% rooting. If this compared to the success of a system in Chile, after 2 years they only managed 4.3 % overall rooting with some individuals reaching 75%.

For the best genetic deployment it is thought that we will have to do a mixture of systems.

CONCLUSION

This lecture has been a broad introduction to tree breeding and genetic deployment using the Western Blue Gum breeding programme for examples. For further information of other breeding and deployment systems a list of a few suggested books and papers from our CALM library is attached.

SUGGESTED READING

Ahuja MR and Libby WJ (1993) Clonal Forestry I. Genetics and biotechnology. Springer. 277 pp.

Ager A (1986) Genetic improvement of willows for energy in Sweden. Report. Swedish University of Agricultural sciences; Section of Energy Forestry. 47 pp.

Butcher TB and Hopkins ER (1993) Realised gains from breeding Pinus pinaster. Forest ecology and management 58: 211-231

Butcher TB and Mazanec RM (1991) Tree breeding in Western Australia: status report. Forest genetics: proceedings of the 11th-meeting of representatives. 42-46

Chaurest PJ and Michel MF (1991) Basics of plant genetic engineering and its potential applications to tree species. Petawawa National Forestry Institute. 48 pp.

Eldridge KG and Matthews CWF (1977). Bibliography of Australian tree breeding and forest genetics to 1975. Divisional report CSIRO, Division of Forest Research. 59 pp.

Fins L, Friedman ST and Brotschol JV (1992) Handbook of quantitative forest genetics. Kluwer Academic 403 pp.

Hopkins ER and Butcher TB (1994) Improvement of Pinus pinaster Ait in Western Australia. CALMScience 1(2): 159-242.

Larsen CS (1956) Genetics in silviculture. Oliver & Boyd, London 224 pp.

Namkoong G, Kang HC and Brouard JS (1988) Tree breeding : principles and strategies. Monographs on theoretical and applied genetics. 180 pp

Shepherd KR (1977) Forest tree breeding in Australia. Divisional report CSIRO, Division of Forest Research. 29 pp

Van Buijtenen JP (1971) Introduction to practical forest tree improvement. Texas Forest Service 17 pp.

Zobel BJ and Jackson BJ (1995) Genetics of wood production. Springer series in wood science. 337 pp

Zobel BJ and Van Buijtenen JP (1989) Wood variation : its causes and control. Springer 363 pp

Zobel BJ, Van Wyk G and Stahl P (1987) Growing exotic forests. Wiley 508 pp.

Zobel BJ and Talbert JT (1984) Applied forest tree improvement. J.Wiley & Sons NY. 505 pp.