

Biotechnology Enhances Utilization of Australian Woody Species for Pulp, Fuel and Land Rehabilitation

JEN A. McCOMB¹, IAN J. BENNETT¹, PAUL G. VAN DER MOEZEL² and DAVID T. BELL²

¹ School of Biological and Environmental Sciences, Murdoch University,

² Department of Botany, the University of Western Australia

Abstract

Selection and micropropagation of superior genotypes of species of Australian genera such as *Eucalyptus*, *Acacia*, *Melaleuca* and *Casuarina* offers a way of capitalizing on Australia's genetic resources without necessarily breaking the tradition of unrestricted export of seed for international forestry. A collaborative program of research and development between Alcoa (Australia), CSIRO Division of Forestry and Forest Products, The University of Western Australia, Murdoch University, North Broken Hill Ltd (APPM Forest Products) and Plantex (Australia) has examined the potential for the use of selected clones of species tolerant to saline waterlogging, and elite selections of *E. Globulus* and *E. nitens* for paper pulp production.

Introduction

Australia is recognized as the home of some of the world's fastest growing hardwood trees, and several genera are widely planted for pulp and fuel in many countries. In Australia there are about 35 million hectares of forested land dominated by eucalypts which form the basis of the hardwood industry giving approximately 1.9 million m³ of timber and 4.5 million tonnes (green weight) of woodchips per annum (Yearbook Australia 1988). Only around 40 thousand hectares are in plantations, but this is about to increase in Western Australia (Shea and Bartle 1988), and in other states, as there is a push towards reforestation to reduce land degradation, improve water quality, provide logs for woodchipping, and allow conservation of native forests (Hawke 1989).

Plantations of eucalypts overseas are estimated at 7 million hectares and far exceed those in Australia (Table 1). Several countries have between 100 and 200 thousand hectares, whilst others have over 250 thousand hectares (Eldridge 1986). Despite

the activity of CSIRO, ACIAR and AIDAB in ASEAN countries, it has been our experience that our neighbours have been turning to Brazil, South Africa, and even Sweden and Finland for genetic material and silvicultural advice rather than to Australia.

The Australian Tree Seed Centre which is part of the CSIRO's Division of Forestry and Forest Products has supplied taxonomically authenticated, high quality seed to researchers and plantation managers in Australia and overseas for 25 years. Seed has gone to over 100 countries. From results of initial trials overseas, seed merchants are then contracted to supply larger quantities of the desired species.

Export of seed from Australia has been free of Government restriction, except for a period when the high-oil yielding species *E. polybractea* was prohibited from export (1944-1953). Despite the fact that many other countries control export of their genetic resources, prevailing opinion is that the wild species are a world resource rather than a national one, and in many cases the difficulty of policing any controls would make restrictions futile. This being the case, how can Australia benefit from our genetic resources which are being so profitably used in other countries?

Countries which utilize Australian plants for forestry, land reclamation, or horticulture have frequently established their industry on a narrow genetic base. In Australia we have access to the entire range of genotypes, and the technology to select and clone elite lines. Surely, as well as exporting the raw genetic material, we could also develop superior lines of the most valuable species and find markets for these improved lines.

The recent legislation for Plant Variety Rights in Australia specifically excludes the registration of a variant found growing naturally in the bush, but it does allow for registration of lines from seed collected in the wild, and on which selection and developmental processes have been applied (Adams 1988, Ellinson 1988). It is thus possible for those who select and develop superior lines of Australian plants to anticipate royalties on the sale of the improved material.

The use of Australian trees for pulp is a major, but not sole aspect, of export of material. The Australian flora has largely evolved under conditions of low nutrient and water availability and is increasingly being exposed to saline waterlogging. The

Table 1. Eucalypt plantation outside Australia. Estimates from Eldridge (1986)

	Area '000 ha	Year of estimate	Main spp
Angola	400	1980	<i>tereticornis</i>
Argentina	100	1973	<i>camaldulensis</i> , <i>viminalis</i>
Brazil	3,000	1984	<i>grandis</i> , <i>grandis</i> × <i>wrophylla</i>
Chile	?		<i>globulus</i>
China	300	1981	<i>citriodora</i>
Congo	?		<i>tereticornis</i> , hybrids
Ethiopia	90	1980	<i>globulus</i>
India	550	1980	<i>tereticornis</i>
Morocco	180	1973	<i>camaldulensis</i>
Peru	90	1980	<i>globulus</i>
Portugal	250	1973	<i>globulus</i>
South Africa	370	1983	<i>grandis</i>
Spain	390	1973	<i>globulus</i> , <i>camaldulensis</i>
U.S.A.	110?	1976	<i>globulus</i> , <i>camaldulensis</i>
Uruguay	110	1973	<i>globulus</i>
Other countries	1,000?		
Total about 7 million			

physiological and structural adaptations that have evolved to withstand these stresses mean that many species are suitable for rehabilitation of degraded land. In establishing trees or shrubs on wasteland the objective may be to simply prevent or slow saline encroachment, but additional benefits are possible in the form of animal fodder or firewood.

A third reason for commercial interest in Australian woody species is for industrial products. At present Australia produces only 5% of the total world production of eucalyptus oil (Lassak 1988) and research is being undertaken to determine whether improvements in yield and growth rate can make plantations of eucalypts in Australia for oil production commercially viable (Brooker *et al.* 1988). Other products such as tannin, rutin and castanospermine face similar problems of commercial viability in Australia but may be exploited in other countries with lower labour cost (Warren 1989). One product that is being developed commercially in Australia at present is melaleuca oil (Southwell 1988).

Table 2. Seedlots despatched from the CSIRO Tree Seed Centre 1986-7 (Data from Midgley 1988)

Genus	Number of Species	Number of seed lots ¹
<i>Eucalyptus</i>	331	6526
<i>Acacia</i>	83	3893
<i>Casuarina</i>	9	758
<i>Allocasuarina</i>	23	194
<i>Melaleuca</i>	31	282
<i>Sesbania</i>	1	74
<i>Grevillea</i>	9	46
Other	84	320

1. In 1987 the geographic regions supplied (% seedlots) were Asia (36.1%), Australia (30.7%), Africa (13.1%) Central and South America (7.2%) Europe (5.7%), South Pacific (3.9%) N. America and the Middle East (1%).

Table 3. Tree Technology Project

Collaborators in the initial phase		
Alcoa of Australia Ltd	Mr. E. David Kabay	coordinator and field trials
The University of W.A.	Dr David Bell Dr Paul van der Moezel	plant screening and field trials
Murdoch University	A. Prof. Jen McComb Dr Ian Bennett	micropropagation research
CSIRO Division of Forestry and Forest Research Canberra	Mr Vic Hartney Mr Stephen Midgley	micropropagation research tree seed centre
Perth	Dr Nick Malajczuk	mycorrhizae
APPM Forest Products North Broken Hill	Dr David de Little	selection of pulp species
Plantex (Australia)	Mr Adrian Bowden	commercial micropropagation

Tree Technology Project

The Tree Technology Project (Table 3) was set up to develop Australia's resources in the first two aspects, high quality pulp species and salt-tolerant species. The expertise of the CSIRO Tree Seed Centre was utilized to select species within the genera

Casuarina, *Eucalyptus*, *Acacia* and *Melaleuca* likely to be salt-tolerant, and then to collect seed from individuals of these species growing in salty areas.

Seedlings were grown to approximately 6 months old by Alcoa (Australia) and they were then screened for their ability to withstand saline waterlogging at the University of Western Australia (UWA). Control pots and salinized pots were drip irrigated with tap water or a saline solution (NaCl : MgSO₄ : CaCl₂ in the ratio of 10 : 2 : 1 by weight) which was increased in strength by 100 mM per week. Waterlogging treatment and waterlogging with saline solutions involved filling the tanks to 1 cm above the soil surface and gradually increasing the salinity as above. The experiment continued until most plants died (usually 2-3 months); the maximum salt level reached was different for the various genera (Table 4). There were 16-24 plants of each provenance of each species in each trial, and this allowed us to select 5 or so individuals from the best provenances. Lines with greatest tolerance to waterlogging, also ranked as superior in the salt waterlogging trial.

Table 4. Tolerance of saline waterlogging of selected species in *Eucalyptus*, *Acacia*, *Casuarina* and *Melaleuca*

	Maximum salt level (mM)	Mean % survival	Mean relative growth ¹	Mean tolerance index ²
<i>Eucalyptus</i>				
<i>camaldulensis</i>	600	75	29	2175
<i>halophila</i>	500	38	12	456
<i>intertexta</i>	500	60	14	840
<i>microtheca</i>	500	80	21	1680
<i>occidentalis</i>	500	59	20	1180
<i>raveretiana</i>	500	68	22	1496
<i>sargentii</i>	500	63	25	1575
<i>spathulata</i>	500	35	29	1015
<i>striaticalyx</i>	500	94	15	1410
<i>tereticornis</i>	500	33	23	759
<i>Melaleuca</i>				
<i>bracteata</i>	900	67	5	335
<i>cajuputi</i>	900	50	0	0
<i>eleuterostachya</i>	900	97	6	488
<i>glomerata</i>	900	75	6	450
<i>halmaturorum</i>	900	100	9	900
<i>lateriflora</i>	900	94	30	2820
<i>lanceolata</i>	900	88	26	2288
<i>quinquenervia</i>	900	32	1	32
<i>thyoides</i>	900	74	23	1702
<i>Acacia</i>				
<i>ampliceps</i>	500	65	42	2753
<i>auriculiformis</i>	500	45	32	1444
<i>aulacocarpa</i>	600	62	0	0
<i>cyclops</i>	600	75	16	1210
<i>hemsleyi</i>	600	47	53	2491
<i>ligulata</i>	600	37	9	333
<i>maconochieana</i>	600	50	0	0
<i>saligna</i>	500	82	17	1387
<i>sclerosperma</i>	600	60	7	420
<i>stenophylla</i>	700	98	92	8993
<i>Casuarina</i>				
<i>glauca</i>	800	100	25	2500
<i>obesa</i>	800	97	46	4462

1. growth (height) of plants treated with saline waterlogging expressed as a percentage of control growth
2. an index computed by multiplying the mean % survival by the mean relative growth

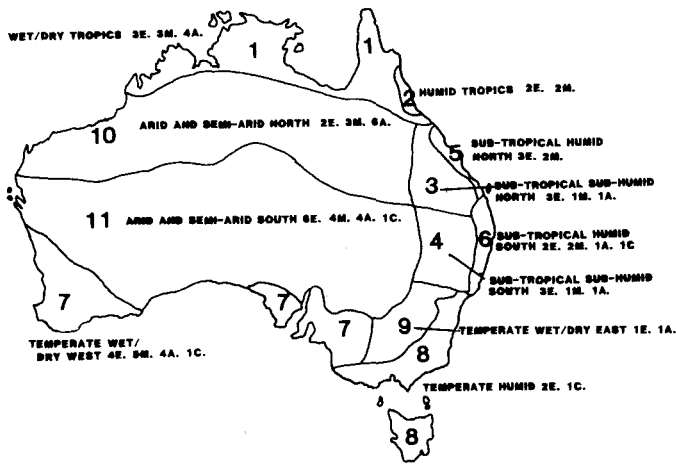


Figure 1. Climatic zones of Australia (Department of Trade and Resources 1982) and the distribution of species tolerant to saline waterlogging selected for micropropagation. *Eucalyptus* (E), *Melaleuca* (M), *Acacia* (A) and *Casuarina* (C).

From successive trials a picture has been built up of the species and provenances within those species that are most salt-tolerant (Van der Moezel and Bell 1989). To compare the results of different trials and different species, the plants were scored on the growth of treated plants compared with controls, and percentage survival (dead plants being those that were permanently wilted or completely necrotic). By multiplying these parameters a 'mean tolerance index' was calculated (Table 4). Within a species the mean tolerance index of the best provenance was of course higher than the mean tolerance in Table 4. The climatic range of the tolerant species was examined and species chosen to cover a wide range of climate zones (Figure 1). Finally the size and habit of the trees, and the uses to which the species could be put, (for example timber, pulp, poles, charcoal, firewood, fodder, windbreaks, shade, shelter, land reclamation, ornamental, honey or oil etc.) was considered to give a selection of around 10 species in each of *Eucalyptus*, *Melaleuca* and *Acacia* and 2 in *Casuarina*.

Screening trials carried out at UWA gave us an indication of the best species and the best provenances within species and we used the 5 or so plants initially selected to work out tissue culture methods. While we did this, a further 200-500 or so plants from the best provenances were screened at Alcoa's glasshouse and the University of Western Australia. From this larger number of plants we selected 20-25 additional plants. For the top 5 species in each genus we aim to have 15-20 clones in culture; from the remaining species 5 or so clones will be set up.

Field trials of salt-tolerant *Eucalyptus camaldulensis* clones have been established in a wide range of saline sites since 1983. In most trials the clones are performing as well or better than the unselected seedlings. More clones of *E. camaldulensis* and other species tolerant to both salt and waterlogging are now being planted, mostly in Western Australia and Victoria, but we have trials in Queensland, Thailand and California. Some of the trials are unmonitored sites on farms, others have been scientifically set up to give information on survival, growth and physiological functioning under saline conditions (Figure 2). A period of 3-5 years will be required to quantify the advantage of planting selected clonal material rather than seedlings, and the effect on the soil.

To obtain maximum growth of eucalypts under conditions of salinity or low soil nutrient status a parallel line of research at the CSIRO laboratories in Perth has investigated the requirement for mycorrhizal fungi and the effect of selection of fungi for

particular soil types (Malajczuk 1987).

The multiplication of plants required for field trials has been carried out by a commercial tissue culture laboratory, Plantex (Australia).

At Murdoch University, work has been completed on micropropagation methods for six species of *Eucalyptus* and five of *Acacia* while at CSIRO Division of Forestry and Forest Products in Canberra methods for seven *Melaleuca* species have been developed. Work is continuing on the remaining species. Although a micropropagation method was worked out for *Casuarina*, other propagation methods were found to be more cost effective (Bennett et al. 1989).

In the case of the pulp species *E. globulus* and *E. nitens*, trees ranked as superior on growth rate and pulp yield have been felled and shoots from coppice growth, cuttings or from grafted plants have been put into culture. The material has been selected by North Broken Hill Ltd (APPM), Forest Resources, W.A. Chip and Pulp, or from *E. globulus* plantations of the West Australian Department of Conservation and Land Management. The micropropagation method for *E. globulus* is successful at research-laboratory level and is being refined for use on a commercial scale. A major concern is to reduce the cost of micropropagated plants by some means of automation (Hartney 1986).

Many countries including some in the ASEAN group have a shortfall of fuel and pulp wood and are experiencing increasing environmental and social problems associated with felling of native forests. Among the range of fast growing or stress-tolerant Australian species of *Eucalyptus*, *Acacia*, *Melaleuca* and *Casuarina* that we have selected and cloned will be many genotypes suitable for plantations in these regions. With the assistance of a grant from Austrade's Innovative Agricultural Marketing Program we have been looking at the potential market for Australian woody species in ASEAN countries, as well as India, the Middle East, Europe and America.

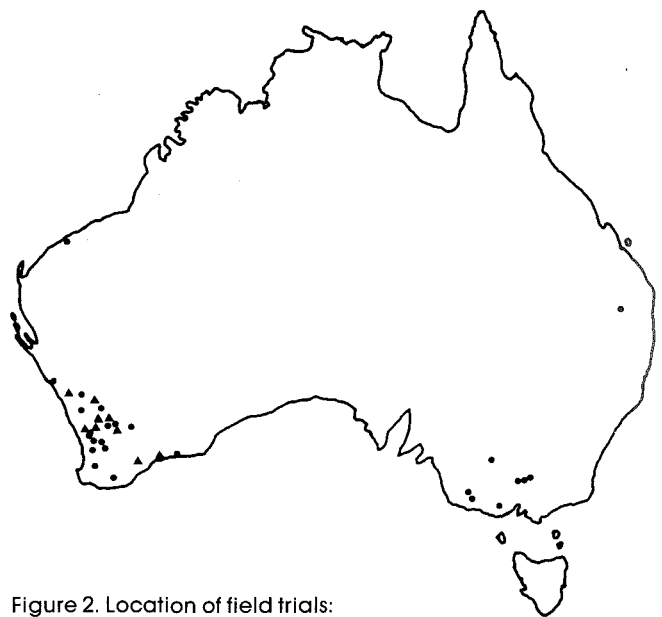


Figure 2. Location of field trials:
● monitored sites
▲ unmonitored sites

There is an awareness that these plantations must not be grown at the expense of highly productive agricultural land, but that they may assist in rehabilitation of mine sites and land degraded by clearing, culling and overgrazing. However, there is a growing feeling that indigenous species must be utilized as well as exotics from Australia. The Tree Technology Research

Group has the capacity to collaborate in the selection, propagation and establishment of plantations of species native to ASEAN countries, or if joint ventures are established by providing training and research facilities. The association with Universities means that training can be tied to postgraduate degrees or diplomas if required. It is our hope that biotechnology can enhance the utilization of Australian woody species for pulp, fuel and land rehabilitation. ■

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References

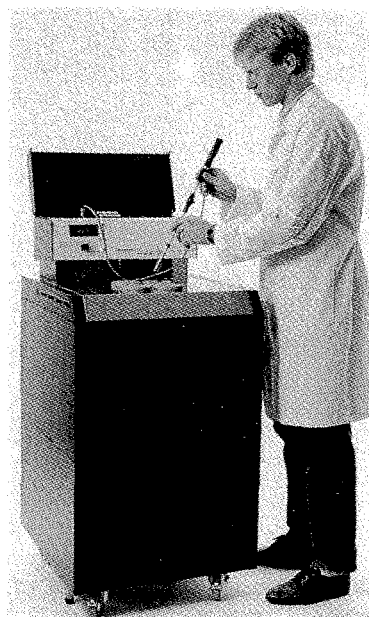
- Adams, K. (ed.) (1988) Plant Varieties Journal 1, on.
- Bennett, I.J., J.A. McComb and C.M. Tonkin (1989) Improvement in rooting of *Casuarina obesa* cuttings with the use of vitamin D2 and D3 (in press).
- Brooker, M., A. Barton, B. Rockel and J. Tjandra (1988) The cineole content and taxonomy of *Eucalyptus kochii* Maiden and Blakely and *E. plenissima* (Gardner) Brooker, with an appendix establishing these two taxa as subspecies. Aust. J. Bot. 36, 119-29.
- Department of Trade and Resources (1982) Australian Farming Systems; an Introduction. Australian Govt. Publ., Canberra.
- Eldridge, K. (1986) Role of Eucalypt Plantations Abroad. Research Working Group No 1, Australian Forestry Council Forest Genetics. Proc. 9th Meeting Bernie, pp. 87-8.

- Ellinson, D (1988) Plant variety rights explained. The Legal View. Aust. Hort. 86(6), 20-23, 68.
- Hartney, V.J. (1986) Commercial aspects of micropropagating *Eucalypts*. VI Int. Cong. Plant Tissue and Cell Culture, Minnesota Abstracts (ed.) D. Somers *et al.* pp 14.
- Hawke, R.J.L. (1989) Our Country Our Future: Statement on the Environment, Aust. Gov. Publ., Canberra.
- Lassak, E.V. (1988) The *Eucalyptus* oil industry, past and present. Chemistry in Australia, Nov 1988, 396-8.
- Malajczuk, N. (1987) Ecology and management of ectomycorrhizal fungi of regenerating forest ecosystems in Australia. Proc. N. Amer. Conf. on Mycorrhizae. Gainesville Florida. pp. 118-20.
- Midgley, S. (1988) The Australian Tree Seed Centre - a window to the resource. Proc. Intern. Forestry Conf. for the Australian Bicentenary, Albury-Wodonga (in press).
- Shea, S. and J. Bartle (1988) Restoring nature's balance. The potential for major reforestation of South Western Australia. Landscape (April), 3-14.
- Southwell, I.A. (1988) Australian tea-tree; oil of *Melaleuca terpinen-4-d* type. Chemistry in Australia, Nov 1988, 480-2.
- Van der Moezel, P.G. and D.T. Bell (1989) Salt land reclamation: selection of superior Australian tree genotypes for discharge sites. Proc. Ecol. Soc. Aust. (in press).
- Warren R.G. (1989) Drug plants - Australia's neglected industry. Aust. J. Biotechnol. 3, 23-6.

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Address for correspondence for this paper: Assoc. Prof. Jen McComb, School of Biological and Environmental Sciences, Murdoch University, Murdoch, Western Australia 6150.

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