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ACTAS

SIMPOSIO LOS EUCALIPTOS EN EL DESARROLLO FORESTAL DE CHILE

Pucón, Chile 24 al 26 de Noviembre de 1993









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ACTAS

S I M P O S I O

LOS EUCALIPTOS EN EL DESARROLLO FORESTAL DE CHILE

(Pucón, Chile. 24 - 26 Noviembre de 1993)

EDITORES:
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PROLOGO

A principios de la década 1960-1970 el Instituto Forestal da inicio a un programa sistemático de selección de especies forestales para las diferentes condiciones climáticas del país. Entre los años 1962 y 1974 se establecen ensayos en más de 60 lugares distribuidos entre las Regiones IV y XII, se prueban en estos más de 160 especies de los géneros **Acacia, Cupressus, Eucalyptus, Pinus, Pseudotsuga** y otras, totalizándose unas 8.000 parcelas experimentales en el programa.

Posteriormente, desde el año 1980 hasta ahora, se ha ampliado esta línea de investigación con numerosas parcelas experimentales complementarias, tendientes a la selección de procedencias de semillas y de técnicas de establecimiento de plantaciones y, más recientemente, al mejoramiento genético de las especies de mayor interés de acuerdo a los resultados de las etapas anteriores.

Tempranamente y en la mayoría de los ensayos, destacan en forma clara diversas especies del género **Eucalyptus**, con rápidos e importantes desarrollos, bajo diferentes condiciones de sitio, aventajando notoriamente a las especies tradicionales utilizadas por los forestadores en el país, **Pinus radiata** y **Eucalyptus globulus ssp. globulus** raza local.

Especies como **Eucalyptus nitens, Eucalyptus delegatensis, Eucalyptus globulus ssp. globulus, Eucalyptus regnans y Eucalyptus viminalis,** en la zona centro sur, y **Eucalyptus camaldulensis, Eucalyptus cladocalyx y Eucalyptus sideroxylon,** en la zona central, empiezan paulatinamente a incorporarse en los planes de forestación de empresas y particulares.

Al progresivo avance en las técnicas de viverización de plantas, de selección de semillas, de establecimiento de plantaciones y a los excelentes crecimientos que resgistran algunas de estas especies, se suman los incentivos estatales a la forestación y la necesidad de incorporar nuevos terrenos a la actividad forestal marginales para el adecuado crecimiento de las especies tradicionales, provocando que las tasas anuales de forestación con especies de eucaliptos se incrementen significativamente, dando inicio a un acelerado proceso de diversificación de

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especies en las plantaciones forestales del país y a la incorporación de nuevos terrenos a la actividad forestal productiva.

Dada la creciente importancia de las especies de **Eucalyptus** en Chile, el Instituto Forestal organiza en el año 1988 un primer seminario técnico sobre el tema, con la participación de especialistas de Australia, Brasil y Sudáfrica y con una importante asistencia de profesionales del sector público y privado, universidades y forestadores particulares. Desde la realización de este encuentro técnico, se desarrolla en el país posiblemente más investigación sobre el tema que en toda la historia de estas especies en Chile, situación que lleva al Instituto Forestal a organizar un segundo seminario al respecto en 1993.

Para este segundo seminario se compromete la participación de especialistas invitados de Australia, Brasil, Francia e Inglaterra y se cuenta también, con la participación de expertos asistentes de Estados Unidos, Sudáfrica, Inglaterra y Argentina. Nuevamente, se cuenta con una importante asistencia de profesionales e interesados del sector y se analizan y discuten 37 presentaciones técnicas, dentro de los temas Mejoramiento Genético, Establecimiento y Manejo de Plantaciones, Plagas y Enfermedades, Forestación en Sitios con Limitaciones Especiales y Utilización.

Los trabajos expuestos en el seminario, en su idioma original, se incluyen editados e in extenso en las presentes Actas, a las cuales también se han incorporado las discusiones de la mesa redonda final.

Los editores agradecen la valiosa colaboración de la Sra. Rosa Somera Quiachón, miembro del cuerpo de Paz de los EE.UU., en la traducción y preparación de algunos textos en inglés, especialmente la mesa redonda final, como asimismo de la Srta. Lorena Larredonda P. en sus labores de secretaria de estas Actas y del seminario.

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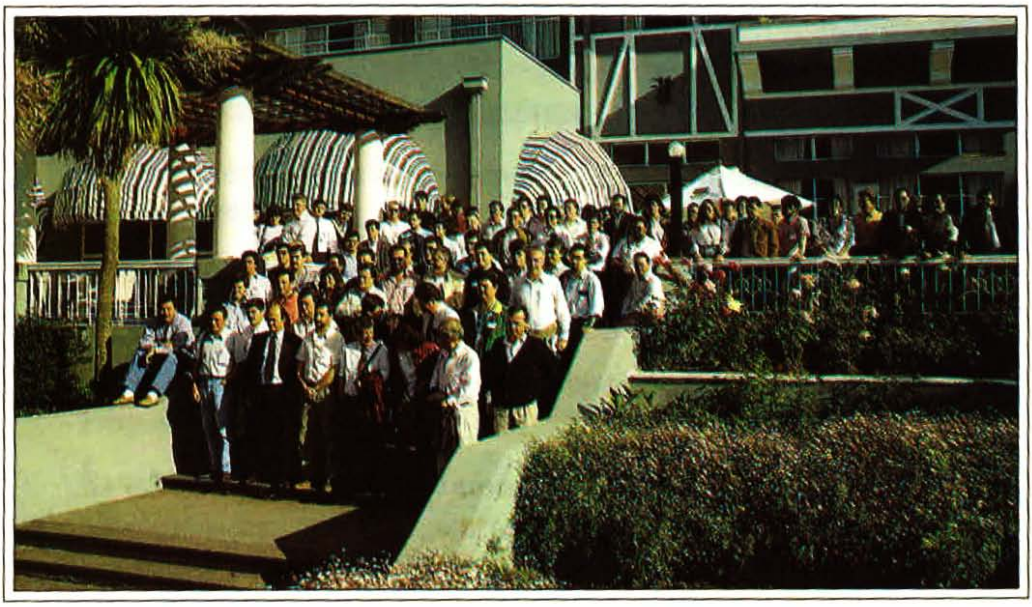

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TEMA I

MEJORAMIENTO GENETICO

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POTENTIAL FOR GENETIC IMPROVEMENT OF EUCALYPTUS IN CHILE

A. R. Griffin (*)

ABSTRACT

The primary level of genetic improvement - appropriate selection of well adapted species and provenances - is essential for all plantation projects. Published results suggest that, in ***E. globulus****, most variation for growth rate is within rather than between provenances. For* ***E. nitens****, patterns are more clearly defined. In Mediterranean climates the most vigorous sources are the Central Highlands of Victoria. Of these, Rubicon and Macalister have greater cold tolerance than Toorongo. However, it is important to appreciate that considerable variation exists between localities within these "provenances".*

For more intensive breeding of improved varieties it is necessary to consider the heritability, variation and genetic correlations of the commercially important traits. In both species growth rate is moderately heritable. Basic density and pulp yield are highly heritable and, while mean density of ***E. nitens*** *is lower than* ***E. globulus*** *there is plenty of scope for improvement through selective breeding. Negative genetic correlations of growth rate with density and pulp yield mean that selection on growth alone is not an adequate strategy if the aim is to improve pulp production per hectare.*

It is important to understand the vegetative and sexual reproductive biology of a species when choosing an improvement strategy. ***E. globulus*** *can be propagated from cuttings, but only about 10% of genotypes root easily (> 70%). If 90% of plus trees must be rejected on this trait alone it is not feasible to make much progress with a simple clonal screening program - the challenge for breeders is to design a program in which rooting evaluation is concentrated in populations which are already superior for commercially important traits.* ***E. nitens*** *is even more difficult to propagate and effort should be focussed on management of improved seed. A major advance in seed production management has been the use of paclobutrazol - up to 30kg seed/ha has been reported for this normally shy-seeding species.*

Several analyses indicate good prospects for obtaining a return from investment in tree improvement. North American experience is that volume gain in the order of 2-6% is all that is required to break even. A detailed analysis of ***E.globulus*** *breeding for an integrated plantation/ pulp mill project estimated total savings in pulp production cost of 15.5% c.f. using unimproved wood.*

(*) Shell International Petroleum Co., Non-Traditional Business Division, Shell Centre, London. SE1 7NA, U.K.

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An operational improvement program is a complex, long-term activity. Experience with the FAMASA program has demonstrated the benefit of working within the framework of a written Strategy Plan. Some major planning issues are outlined, and the importance of long-term management commitment is emphasised. Finally, it is noted that the benefits of breeding will be wasted unless excellent silvicultural practices are adopted (and viceversa).

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INTRODUCTION

Around the world it is increasingly clear that investment in genetic improvement can add both quantity and quality to the wood produced from plantations of **Eucalyptus** (see Eldridge **et al.**, 1993). A number of organisations in Chile are already applying tree improvement technology (Jayawickrama & Balocchi, 1993) and others are no doubt contemplating such an investment. In this paper I will review opportunities for improvement, focussing particularly on **E. globulus** and **E. nitens**, which have emerged as the major species for industrial wood production in this country; present some examples of the economic benefit of investing in tree improvement; and conclude with comments on key aspects of planning and implementation. This discussion draws heavily on the experience gained in working with colleagues responsible for the Fibre Yield Improvement Program (FYIP) of Forestal y Agricola Monte Aguila SA (FAMASA).

A review of the current status of **Eucalyptus** improvement in Chile is outside the scope of the paper, as is any detailed treatment of the theory and/or technical procedures which must be mastered in order to actually implement an FYIP.

GENETIC VARIATION AVAILABLE FOR USE BY PLANTATION GROWERS

In common with almost all tree species, the Eucalypts are at a very early stage of domestication and enormous genetic variation exists between species and between and within provenances. Since the largest, cheapest and fastest gains are to be made by ensuring that plantations are established using the best available species and seed sources within species (Zobel & Talbert, 1984), it is hard to visualise circumstances where it would be unprofitable to invest in at least this primary phase of tree improvement.

In order to move on to more sophisticated programs for breeding new improved varieties we need to understand the heritability of commercially important traits and the way in which genetic variation is distributed within and between populations. Such knowledge comes mainly from field evaluation trials but it is not necessary for every organisation to "re-invent the wheel". Breeders must make sure they use the increasing amount of information which is available in the international literature.

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Selection of Species and Provenances

The long-term history of successes and disasters in European plantation forestry led Anderson (1961) to the following principle: "the only safe basis for choice of species, especially in the creation of new forests, is the purely ecological one... Where this (adaptation to site) is thought to apply equally to two or more species, the final choice may then be based in the expected value, not volume, production of the various species".

In practice, it is all too easy to make a premature assumption that it will be possible to grow those species which are most favoured by the current market - in Chile the obvious example being the planting of **E. globulus** on sites which are too cold. Tree breeders must be prepared to argue this point in the interests of the long-term sustainability of plantation programs - but must of course present managers with acceptably well-adapted options. It is worth noting that the dominance of one conifer species (radiata pine) in the plantations of Australia and Chile is something of an exception in world forestry. The more usual case is that several species are grown to take advantage of environmental variation within a plantation estate. Perhaps we should not expect to find a single "wonder species" in the genus **Eucalyptus**, where species generally occur in nature in mosaics and mixtures rather than mono-specific stands.

Through the activities of INFOR, there has already been quite comprehensive evaluation of Eucalypt species in Chile (Anon, 1986). As elsewhere in the world, species of the sub-genus **Symphyomyrtus** are proving most reliable, though the **Monocalyptus** species **E. delegatensis**, **E. regnans**, and **E. fastigata** are capable of good growth on some sites. For production of pulpwood in Region VIII, FAMASA regard **E. globulus ssp. globulus** as the primary species, with **E. nitens** favoured on the colder sites, where **E. viminalis** and **E. smithii** are also plantable as pure species. In the FYIP an even broader range of species is evaluated because these may contribute useful genes in hybrid combinations.

It is important to emphasize the point that crop value, not volume, should be the basis for choice in any program. As an example, Table 1 shows unpublished data from Riocell (Brazil) indicating the importance of wood density and pulp yield - although **E. dunnii** has a lower MAI than **E. grandis**, it is actually capable of producing a higher yield of pulp/ha/yr.

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Table 1.

PRODUCTIVITY OF EUCALYPT SPECIES IN RIO GRANDE DO SUL, BRAZIL AT 7 YEARS (Riocell unpubl.)

Species	(A)Basic Density	Unbleached Pulp Yield (%)	Production/ha/yr		
			(B) MAI (m^3)	Fibre Yield (AxB)	Pulp (BDT)
E. grandis	0.42	52	42	17.64	9.17
E. saligna	0.46	50	36	16.74	8.37
E. tereticornis	0.55	46	25	13.48	6.20
E. maidenii	0.54	53	32	17.28	9.16
E. dunnii	0.52	53	35	18.20	9.64

Patterns of natural (provenance) variation: E. globulus ssp. globulus

There is surprisingly little published data on provenance level variation in this species. The earliest range-wide collection, made by Orme in the mid-1970s, has been quite widely tested in Australia and elsewhere. Tests over 2 trial sites in Tasmania failed to detect statistically significant provenance variation in growth at age 7 (Volker & Orme 1988). Subsequently CSIRO Australian Tree Seed Centre made a more comprehensive collection and data from worldwide plantings are currently being analysed with the support of the Southern Tree Breeding Association (Australia). Two-year growth data from one of these trials in NW Tasmania has recently been reported (Potts & Jordan 1993). Highest performing sub-provenances and families were concentrated in three main geographic regions - the Otway Ranges (VIC), Jeeralang/Strezlecki Ranges (VIC) and south of Geeveston (TAS) (see Figure 1). This is reasonably consistent with the pattern of variation observed by Volker & Orme (1988) at a similar site and by Spencer & Williams (1993) who are testing part of the CSIRO collection in southern Victoria. Eldridge **et al.**, (1993) note that, where seedlots of landraces from several countries have been tested, they have shown approximately the same average growth as wild provenance samples.

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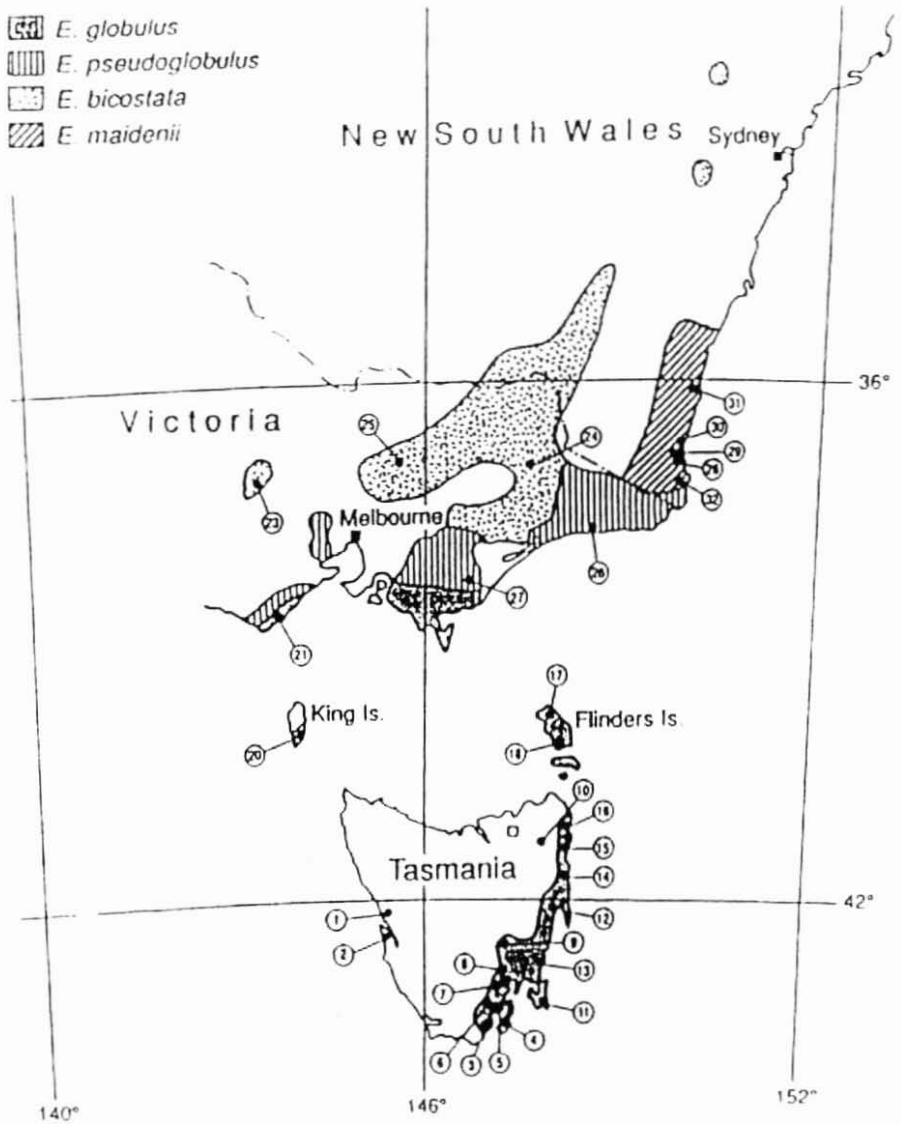

Figure 1. **NATURAL DISTRIBUTION OF THE FOUR SUB-SPECIES OF Eucalyptus globulus SHOWING LOCATION OF SITES FOR THE ORME COLLECTION (Volker and Orme, 1988). SEEDLOT 21 IS IN THE OTWAYS REGION; 27 THE JEERALANGS; AND 3 THE AREA SOUTH OF GEEVESTON, TASMANIA.**

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Intra-specific variation in resistance to environmental stresses and/or pests and diseases can sometimes be exploited at the provenance level.

Mycosphaerella leaf disease is a problem on some coastal sites in Chile and Carnegie **et al.** (1993) have reported that sub-species and provenances of **E. globulus** S. lat. do show some variation in susceptibility. In a trial in Victoria, defoliation ranged from 32% for ssp. **globulus** from the Otways (VIC) to 9.9% for trees of ssp. **maidenii**.

Lack of cold tolerance is certainly a major constraint on use of **E. globulus**. Tibbits **et al.**, (1991) have shown that the mean freezing resistance of hardened seedlings of the most and least tolerant provenances differs by 1.4°C but, since cold stress was not a problem in any of the reported field growth trials, we cannot judge whether the more hardy provenances are more productive on colder sites. It is important to test whether evaluation in such environments does increase provenance x site interaction effects. However, since the species has been widely planted for some decades, it is reasonable to assume that silviculturalists would have reported any radical successes or failures with different seedlots - and therefore that provenance main effects and/or interactions are not as substantial as in many other species of **Eucalyptus**. If this is true, the breeder is free to concentrate on exploitation of variability within the full range of provenances.

Patterns of natural (provenance) variation: E. nitens

Economically important provenance variation and provenance x environment interactions are much more clearly established in **E. nitens** (Figure 2). The first field studies by Pederick (1979) established the inferior growth potential of Errinundra provenance (now raised to specific status as **E. denticulata**, Cook & Ladiges, 1991) and suggested that, for cool temperate planting sites with predominantly winter rainfall, populations from the Central Highlands of Victoria were higher yielding than those from New South Wales. The high productivity of the Central Highlands provenances was confirmed by Kube (1993) in Tasmania, although in his 10-year-old trials they were not significantly better than southern NSW (Table 2). It is important to note the large variation in growth potential within the Central Highlands provenances. For example the highest volume produced in the Tasmanian trials (191 $m^3 ha^{-1}$) was from Mnt. Skene (Macalister), but Mnt. Wellington (also Macalister) produced only 145$m^3 ha^{-1}$. Care must therefore be taken with the terminology used by Australian seed collectors as provenance names such as "Toorongo", "Macalister" and "Rubicon" related to regions rather than particular localities - and it is the latter which should be specified if you wish to repeat an order of a good seedlot. Some

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Toorongo populations also contain a mixture of the **E. denticulata** type and these are best avoided as such phenotypes are less cold tolerant than the normal "juvenile persistent" form (Raymond **et al.**, 1992) and may contribute to a more variable stand structure.



Figure Nº 2. **NATURAL DISTRIBUTION OF Eucalyptus nitens. LOCATION OF THE OUTLYING NORTHERN NEW SOUTH WALES POPULATIONS ARE SHOWN IN THE INSET (After Pederick, 1979).**

Table Nº 2.

GROWTH OF E. nitens SEEDLOTS OVER 2 SITES IN TASMANIA AT AGE 10 YEARS (After Kube, 1993).

PROVENANCE	Nº LOCALITIES EVALUATED	VOLUME ($m^3 ha^{-1}$)	
		PROVENANCE MEAN	RANGE OF LOCALITY MEANS
Toorongo	5	165.7	142.3 - 181.4
MacAlister	3	164.5	145.4 - 191.2
Rubicon	5	161.2	135.4 - 176.8
S NSW	3	160.2	156.4 - 163.2
Errinundra	3	111.7	111.5 - 137.3
N NSW	1	90.3	
LSD (P<0.05)		30.0	38.1

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E. nitens also shows substantial site x region of provenance interaction. Growth of NSW provenances has proved better than those from Victoria at sites with moderate summer rainfall in South Africa (Purnell & Lundquist, 1986). This is attributed to their greater resistance to **Mycosphaerella** leaf fungi.

Provenance variation in frost hardiness has been reported by Tibbits & Reid (1987) and also Raymond **et al.**, (1992) who assayed seedlings from 198 families from throughout the range and found that provenance accounted for up to 29% of total variation. Northern NSW was most frost tolerant and Toorongo the least. Rubicon, Macalister and southern NSW were intermediate and not significantly different from each other. The Errinundra provenance was least frost tolerant of all populations studied by Tibbits & Reid (1987). This pattern of genetic variation in hardiness could explain differences in early growth rates observed by FAMASA (unpubl.). Trees of Toorongo origin are growing best in yield trials on coastal sites but on colder sites inland, the fastest growing seedlot is from Macalister.

Genetic parameter estimates for some commercially important traits in E. globulus and E. nitens

In order to plan more intensive breeding programs and to predict resulting expected gains, it is necessary to have at least rough estimates of mean, variance and heritability of each trait together with genetic correlations between traits. The literature is increasingly valuable as a source of first approximations and some published examples for **E. globulus** and **E. nitens** are summarised in Tables 3 and 4.

In inspecting the data in Table 3 one is struck by the considerable similarities across the range of trials. Heritabilities for growth are moderate, with estimates generally somewhat higher for height than diameter. Of the form/branching traits, stem straightness has moderate heritability in both species but there is lack of concensus about branch size which is probably ascribable to test conditions (Whiteman **et al.**, 1992).

Basic density is quite highly heritable in **E. globulus** as is pulp yield.

Reports of correlation estimates are too detailed for easy summary. An important finding by both Borralho **et al.**, (1993) and Dean **et al.**, (1991) is that the genetic correlations of volume with basic density and pulp yield are slightly negative. Selection on volume alone is therefore not an adequate strategy if the aim of the FYIP is to enhance pulp production.

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Table Nº 3.

SOME HERITABILITY ESTIMATES FOR E. globulus (EG) AND E. nitens (EN).

Volker et al (1990) — 6 year old open pollinated EG progeny of multi-provenance origin in Tasmania

Trait	h^2 ± s.e
Height	0.12 0.05
Diameter	0.24 0.07
Stem Form	0.22 0.07
Branch Size	0.29 0.08

Borralho et al (1992) - 3 open-pollinated EG progeny trials of varying origin in Portugal (only latest estimates summarised (age in parentheses))

Trait	h^2 ± s.e		
	Trial 2	Trial 3	Trial 4
Height	0.17 ± 0.06 (4)	0.29 ± 0.19 (8)	0.33 ± 0.19 (9)
Sectional Area	0.13 ± 0.05 (18)	0.15 ± 0.04 (6)	0.16 ± 0.10 (8)

Dean et al (1991) — Sub-set of trees in same EG trial as Volker et al (1990), assessed at age 8

Trait	h^2 ± s.e
Height	0.22 ± 0.16
Diameter	0.17 ± 0.15
Basic Density	0.78 ± 0.22
Pilodyn	0.21 ± 0.16
Pulp Yield %	0.56 ± 0.20

Borralho et al (1993) - Heritability and additive genetic and phenotypic correlation estimates (age 12) used in modelling breeding objectives for EG

Trait	h^2	Correlations*		
		VOL	DEN	PULP
Volume	0.20		-0.10	-0.05
Density	0.65	-0.10		0.30
Pulp yield %	0.30	-0.10	0.30	

* Additive genetic correlations above the diagonal, phenotype correlations below

Whiteman et al (1992) - 9 year old open-pollinated progeny trial of EN from central Highlands provenances, in Victoria

Trait	h^2 ± s.e
Height	0.23 ± 0.07
Diameter	0.18 ± 0.06
Stem Straightness	0.20 ± 0.05
Branch angle	0.11 ± 0.05
Branch thickness	0.04 ± 0.03
Branch retention	0.21 ± 0.07

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Table Nº 4.

SOME ESTIMATES OF WHOLE TREE BASIC DENSITY FOR POPULATIONS OF a) E. globulus b) E. nitens.

SOURCE		Age (yrs)	Mean B.D (kg m $^{-3}$)	± S.D
a)				
Borralho et al (1993)	Estimate used by CELBI for modelling	12	560	40
Valente et al (1992)	55 tree sample by PORTUCEL	12	550	30
Dean et al (1991)	194 tree multi provenance sample, Tasmania	8	482	30
Orme (1992)	b.h core samples	9	462	21*
FAMASA (Unpubl.)	48 tree sample	8	535	29
b)				
McKim & Ilic (1987)	5 tree x 5 provenance sample, Victoria	8.5	480	28*
Purnell (1988)	5 tree x 5 provenance sample, South Africa	11.5	512	30*
Orme (1992)	b.h. core samples	9	434	20*
FAMASA (Unpubl)	48 tree sample	8	457	30

* Estimated from published range or S.E. data

An additional factor which is important to quantify is age-age correlation (because rate of gain is maximised if selections are made as early as possible). Borralho **et al.**, (1992b) drew the important conclusion that selection on height when the trees were **ca.** 8 m tall (about age 4 under Portuguese conditions) almost doubled the genetic gain obtained **cf.** direct selection on growth at age 8 years.

Density is an extremely important determinant of pulp and paper properties of eucalyptus wood (Arbuthnot, 1991; Vasconcellos Dias & Claudio-da-Silva, 1985) and is a trait for which **E. globulus** and **E. nitens** differ rather widely. Some examples of population means and standard deviations are summarised in Table 4.

Absolute values are not directly comparable across trials because of age, site and differences in sampling procedure, however two key points emerge: In all trials there was substantial variability which, combined with the high heritability (Table 3), leads us to expect good success in changing the population mean through

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selection. Secondly, the distributions of the two species clearly overlap and relatively higher selection pressure on density in **E. nitens** could be used to make plantations of the two species converge for this trait.

BIOLOGICAL AND TECHNICAL CONSTRAINTS ON CAPTURE OF GENETIC GAIN

The genetic variability described above clearly offers an opportunity to improve the productivity of Chilean eucalypt plantations through breeding. There are however a number of biological characteristics of the species which must be understood and managed if this opportunity is to be realised in practice.

Propagability

The major success story in rapid improvement in **Eucalyptus** has undoubtedly been the Aracruz clonal selection program (Brandao **et al.**, 1984). Unfortunately this simple approach of phenotypic selection and screening has not proved widely practicable because most eucalypt species will not root easily on an operational scale. This is certainly true of **E. globulus** and **E. nitens**. Haines & Woolaston (1991) have shown that loss of potential realised genetic gain becomes increasingly serious as the proportion of selected genotypes which can be utilised declines. This is because selection pressure has to be placed on propagability at the expense of traits with direct commercial value.

It is difficult to compare data on rooting success as cultural treatments and/ or the degree to which the propagated population has already been selected for rooting ability, are often unclear. Trends are, however, apparent from the information assembled in Table 5.

For a population of **E. globulus** genotypes of either seedling or coppice origin, selected without regard to rooting ability, it seems reasonable to assume that about 10% will strike with over 70% success. Once such a population of good rooters has been identified then it is possible to repeat the result on an operational scale - as shown by the Portuguese company SOPORCEL.

Although **E. nitens** has a reputation for being much more difficult to propagate, this view is not supported strongly by the small amount of published data. In the heritability study reported by de Little **et al.** (1992) average rooting was 15% **cf.** the 20% in the similar study of Borralho & Wilson (1994) with **E. globulus**. Also in the in vitro rooting studies of Willyams **et al.** (1992) 3.4% of clones had >70% rooting capacity **cf.** 5.3% for **E. globulus**. It is the opinion of APPM (de Little **et al.**, 1992) that mass micro-propagation is the way forward with **E. nitens**.

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To grasp the scale of the problem in applying the Aracruz approach directly to **E. globulus**, consider the following data from Carton de Colombia (Lambeth **et al.**, 1991) with the "easy" species **E. grandis.** 1100 plus trees were selected, and 460 (42%) coppiced and rooted sufficiently well to field test, giving 65 (6%) clones selected for superior growth and form (Note that the select population would be further reduced if wood/pulp properties had also been taken into account). With the same retention rates at the coppicing and clonal evaluation phases, but with high rooters only occurred at a frequency of 10%, we would need to screen an initial population of 4600 **E. globulus** plus trees in order to produce an equivalent 65 useable selections.

Table Nº 5.

SOME INDICATIONS OF ROOTING SUCCESS FOR a) E. globulus b) E. nitens.

SOURCE		% Population tested
a)		
Wilson (1992)	Clones from coppice or seed with good rooting ability (>70%)	5-10
Borralho (1994)	Propagation success of cuttings from seedling population un-selected for rooting ability	20
PORTUCEL (1993 unpubl)	Good rooting clones from select plus trees	10
SOPORCEL (1993 unpubl)	Plantable plants from operational nursery using 10 good rooting clones	65
Willyams et al (1992)	% of 700+ seedling derived clones with in vitro rooting >70%	5.3
de Little et al (1992)	Long term average rooting % of 2 clones as cuttings	45.4
de Little et al (1992)	Long term average rooting % of same clones in vitro	53.4
Shell (1993 unpubl)	% of clones of seedling origin with >70% rooting in glasshouse trials in U.K	12
b)		
de Little et al (1992)	Average rooting of 600 seedling derived clones	15
Willyams et al (1992)	% of 600 + seedling derived clones with in vitro rooting >70%	3.4

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This analysis is not intended to discourage development of vegetative propagation programs for this species. Some genotypes clearly can be propagated easily, and the challenge is to develop strategies which will ensure that we focus the search for these in populations which are already highly select for production traits. A number of options available (see e.g. Haines & Griffin, 1992) - and in the longer term, since rooting is quite highly heritable (h^2= 0.41 ; Borralho & Wilson 1994), the problem could be reduced if we adopt breeding strategies which increase the frequency of these genes in the breeding population. In the short term, a useful approach adopted by CELBI is to screen for good rooting genotypes within proven high yielding families (Wilson, 1992).

The current FAMASA and Shell view is that we will focus resources on mastering the propagation of **E. globulus** and regard **E. nitens** as a seed-propagated species for the short to medium term. However, in the longer term it is desirable that operational vegetative propagation is developed for all intensively managed tree species - otherwise it will not be possible to access the additional genetic gains which are becoming available through new biotechnology (Purse, this Conf.)

Flowering and Seed Production

In estimating genetic gain available via improved seed it is implicit that all select genotypes can be mated with all others at predictable frequencies in seed orchards and/or controlled crossing programs, and that sufficient seed is produced to satisfy demand for nursery stock. Deviation from this ideal can lead to loss of gain for the same reasons as described for vegetative propagation - though the problem may be less obvious (nobody can see the difference between bags of seed capable of producing 25 and 30m^3 ha^{-1} MAI).

A comprehensive review of reproductive characteristics of Eucalypts is provided in Griffin (1989). Here I will comment only on the main species of interest.

One of the early decisions in planning an FYIP relates to the area of seed production facility required to meet a given demand for seed. Based on our examination of yield from trees growing in Region VIII, and experience in the few other orchards around the world, we assumed for the FAMASA program that we would obtain 10kg/ha/yr uncleaned seed from clonal seed orchards of **E. globulus**, and 3kg/ha/yr from seed production areas and seedling orchards of **E. nitens**. These estimates are now considered very conservative as experience is gained in the use of paclobutrazol to enhance production (Griffin **et al.**, 1993; Rojas, this Conf.).For example, treatment has recently increased yield in an **E. nitens** SSO of APPM by an order of magnitude, to around 30kg/ha (Tibbits pers.comm.). There is no reason to think that **E. globulus** will be any less responsive.

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The management of **E. globulus** orchards with selections from multiple provenances does present one unusual challenge: this winter/spring flowering species can flower over a long period and there is a substantial component of provenance variation. In an orchard in NW Tasmania, trees from SE Tasmania flowered on average 50 days earlier than those from Bass Strait provenances (Volker, unpubl). Trees with extreme flowering times are therefore highly likely to cross only with selections from their own provenance or even family (with undesirable inbreeding consequences). It is important to assess whether this problem persists in the Chilean environment, and if so to consider whether phenology should be taken into account when culling orchards.

All eucalypts are pollinated by animals and it is important to ensure that adequate vector populations are present when species are grown in any exotic environment. The flower-feeding insects present in Chile during the summer months are probably adequate to pollinate **E. nitens**, but the situation with **E. globulus** is less clear - the birds which contribute to its pollination in Australia are not present, and only large insects such as honeybees are effective pollinators. It cannot be assumed that these will be present at high frequency at flowering time and FAMASA are currently experimenting with the introduction of hives to their clonal orchard.

The greatest rates of genetic improvement are achievable when breeding populations are advanced by controlled of rather than open-pollination. Controlled-pollination is also the only practical short-term means of producing inter-specific hybrids. Both CELBI (for **E. globulus**) and APPM (for **E. nitens**) are advancing their breeding populations by this means and CELBI is even producing significant quantities of high quality full-sib seed for direct use and/or multiplication by vegetative propagation. Any organisation aiming to develop an intensive eucalypt improvement program needs to master this technology.

E. globulus is an easy species to cross at the intra-specific level. The flower buds are large, robust and not given to abortion; of up to 100 seeds can be produced in a single capsule. Unfortunately it is not currently possible to use it as a mother plant in making crosses with other smaller-flowered species (Potts & Savva, 1989), so any hybridisation program must either be carried out in collaboration with breeders of the alternative species or incur the additional expense of a multi-species evaluation/selection/arboretum management program. The hybrids must show correspondingly greater superiority in order to justify these costs.

Other Technical Constraints

Techniques which are useful in demonstrating the existence of genetic variation on an experimental scale may be impracticable in an operational breeding program.

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Two main problems may occur: for stress or pathogen tolerance traits it is difficult to control the test environment so that variation is expressed reliably of while still retaining a plant population of appropriate size. A reliable technique for operational screening for relative frost hardiness has been developed in Australia (Raymond **et al.**, 1992), but there are no comparable tests for other physiological traits such as drought tolerance or susceptibility to leaf fungi. An allied problem is that, although a trait may have great economic weight, assays are so expensive that only a small number of samples can be examined. De Little **et al.** (1992), identified screening for pulp yield as the major bottleneck in the APPM program and stated that a reliable non-destructive method must be found. Indirect assay of correlated traits such as cellulose content, through use of NIRA (Near Infra-red Reflectance Analysis) (Garbutt **et al.**, 1988) shows most promise of a breakthrough.

It is hopefully clear from the above sections of that an operational FYIP requires the support of an ongoing R&D effort. The biological and technical complexities of breeding are such that there will always be bottlenecks preventing the maximisation of genetic gain - and removal of one bottleneck will result in some other factor becoming limiting. An appropriate allocation of investment to breeding and supporting research is an ongoing challenge for FYIP managers.

SOME INDICATIONS OF ECONOMIC BENEFIT FROM GENETIC IMPROVEMENT

From analysis of genetic experiments it is possible to predict the changes which can be made in plantation mean values for the trait(s) under selection. However, the $ value to the grower is rather specific to management objectives, scale of plantation estate, and probability of actually realising a premium for any increases in quality traits. For example, a company growing wood for production of pulp in its own mill may find it highly profitable to breed varieties with higher pulp yield - but a tree grower aiming to sell into a local market would probably emphasize volume production alone.

Even though we cannot generalise about the precise benefits of genetic improvement the following set of examples, approaching the issues from different perspectives, show that managers can have great confidence that a well planned program will prove an attractive investment.

1) Zobel & Talbert (1984) cite several examples of North American analyses which have considered the amount of genetic gain required to justify the cost of a tree improvement program. General conclusions for these of conifer programs are that it is necessary to produce volume yield increases in the order of 2-6% **cf.** use of alternative unimproved seed sources. These are modest gains compared with

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those expected for eucalypts, where return on investment is also achieved earlier because of the shorter rotations. If value can be added by associated improvement in quality traits, then expenditure on an FYIP will be justified even more easily.

2) Borralho **et al.** (1993), projected genetic gains from the CELBI **E. globulus** improvement program in Portugal and then calculated the impact on unit cost of kraft pulp production for a company with a mill capacity of 250,000t and self-sufficiency for wood supply. Cost components considered were plantation establishment, maintenance, harvesting, transport and operational mill costs. Use of seed from a unculled 1st generation orchard increased volume production by 15% and reduced total pulp production cost/tonne by 2.8%. Following selection on an index which included volume, density and pulp yield, the second generation improved crop was estimated to reduce total production costs by 15.5% **cf.** the unimproved plantation - equivalent to an annual increase in profit of US$ 6.68 M at 1991 prises.

Dean **et al.** (1991), conducted a similar analysis for the APPM mill plantation project in Australia, using genetic data from an 8-year-old progeny trial of **E. globulus**. Predicted combined wood and mill production cost savings were 22.5% of current mill gate wood costs (expressed on this basis the Borralho **et al.** (1993) saving was 27.4%).

3) Consider the cost premium which you could justify if you could be sure that a particular seedlot would yield just 1m^3 MAI more than the best alternative (e.g. increasing MAI from 25 to 26m^3/ha/yr, which is a gain of 4%). The following assumptions are reasonable for **E. globulus** in Chile:

1 kg of clean seed will produce enough plants for 200 ha.
Trees are grown on a rotation of 10 years.
Present Value of wood at stump is US$ 12/m^3

1 kg of the best seed will therefore yield an extra 2000 m^3 of wood, worth $24000. This represents the break-even premium which could be paid for the seed (ignoring any expenditure in evaluating which seedlot to purchase).

The substantial margin over the current prices of $US 100-300/clean kg for Chilean seed and around $US 1300/clean kg for Australian seed of reliable provenance, could be spent on yield testing and/or breeding without reducing the profitability of the plantation project. Still greater gains are of course expected from more intensive breeding (e.g. around 9 m^3 MAI increase is projected for the CELBI D95 program) - further increasing the level of expenditure which can be justified for the necessary FYIP work program.

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4) Estimates of relative NPV of increases in volume production through different improvement options for pulpwood crops of **E. globulus** in Australia are shown in Table 6. The importance of focussing on gain per unit time rather than per generation is emphasized - for example mass-propagation of families (option 4) would be profitable than waiting a further 4 years in order to identify and clone the very best individual genotypes (option 5). In general, options which produce greatest gain per generation take the longest time and are more costly to implement - the marginal net value of additional genetic gain is thus an important project-specific determinant of FYIP strategy.

Table Nº 6.

GENETIC GAINS EXPECTED FROM REALISTIC IMPROVEMENT OPTIONS FOR SOUTHERN BLUE GUM IN AUSTRALIA (Griffin et al., 1990)

Option		Percentage Gain cf Option 1	Lead Time (planting stock first available) (yr)	Indicative net benefit of gain (volume only) ($/ha)
1	Unimproved seed	0	1	
2	Culled seedling seed orchard (SSO)	7.5	6	227
3	Culled clonal orchard (CSO)	14.4	8	402
4	Mass propagated control-pollinated families of outstanding parents	22.5	9	556
5	Tested clones from control-pollinated families	25.7	13	484

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SOME THOUGHTS ON PLANNING FYIP ACTIVITIES

This review clearly demonstrates the scope for enhancing crop value through genetic improvement. It is easy to say that the breeder simply needs to identify the best trees and use those to produce seed or cuttings for use in the plantations - but somewhat more complex in practice!. I will conclude with some comments on planning and implementing a work program, using the FAMASA FYIP as an example. The defined goal of this program is to:

> "Maximise the rate of genetic improvement in yield, quality and uniformity of fibre production while recognising the need for rapid adaptation to changing silviculture, harvesting and processing technologies, and market opportunities".

Any tree improvement program has of two parallel activity streams (Figure 3). The Breeding Cycle - which may be repeated for an indefinite number of generations - involves the testing, selection and inter-breeding of the most desirable genotypes. Benefit from this effort is Captured by using the best material currently available to produce operational planting stock. Depending upon the biology of the species and resources available to the program, this may be achieved through seed orchards or vegetative propagation with (in future) additional value added through Genetic Modification technology.

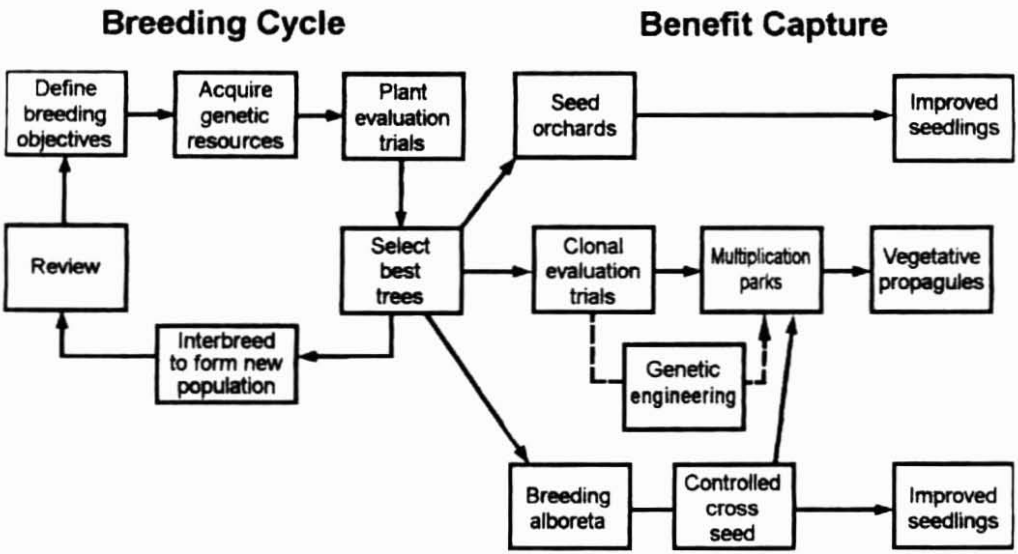

Figure Nº 3. **OUTLINE OF A TRE IMPROVEMENT PROGRAM. THE BREEDING CYCLE CAN BE REPEATED INDEFINITELY, GIVING CUMULATIVE GENETIC GAIN. THE BENEFITS OF EACH GENERATION OF BREEDING ARE CAPTURED AS SUCCESSIVELY HIGHER VALUE GENOTYPES BECOME AVAILABLE FOR SEED ORCHARDS OR VEGETATIVE PROPAGATION.**

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For each step in each activity stream there are technical skills required and many options to consider. Information will often be scarce, or not available until some preliminary work has been carried out.

Some examples of major questions we faced when the FAMASA FYIP program commenced in 1989 where:

-Should the **E. globulus** breeding population be based on Australian material, the Chilean landrace (or both?).

-What weight should be placed on evaluation of alternative species?

-What range of sites should be covered by evaluation trials (at a time when the company's land acquisition strategy was not fully developed)?

-Should we be breeding hybrids rather than just improving the pure species?

-Would the main route for benefit capture be through improved seed or vegetative propagules?

-Should expertise in quantitative genetic analysis and wood/pulp evaluation be developed in-house or contracted in?

The general planning principle adopted was that we should attempt to answer questions requiring R&D as quickly as possible and, where the way forward is unclear, to delay decision-making as late as possible (so that the information base is maximised). This has meant accepting of the fact that parts of the work program will become redundant over time, as the price for minimising situations where we will have to back-track and sacrifice gain per unit time.

To take the example of benefit capture strategy. We started with an assumption that vegetative propagation technology would be available by the time that we had made selections in the 1st generation breeding population trials. However a minimum 3 year program was required for FAMASA to adapt and scale up the techniques developed by Shell in U.K. Rather than lock in solely to this option it was decided to start the R&D program in parallel with establishment of enough Clonal Seed Orchard to satisfy company seed requirements from 1996 onwards. By late 1994 the company will be in a position to refine its view on the optimal way forward.

With so many FYIP activities developing at different rates, and with different degrees of technical difficulty and risk, it is highly desirable to produce a written Strategy Plan (SP). The FAMASA SP has a 10-year time horizon and addresses the following issues:

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-Schedules for each activity in the breeding cycle - including time frames for acquisition of necessary technical skills/information (e.g. by the time progeny trials are old enough for assessment and selection it will be necessary to have in place the capability to carry out the genetic analysis and also to have worked through the problem of economic weighting of different traits with colleagues in management).

-Schedules of benefit capture activities and projected plant supply from the increasingly improved sources (management planners need to be able to take the fruits of your labours into account!)

-Milestones for review of progress with the different activities - although I have stressed keeping multiple options, it is essential to ensure timely decisions in the interests of cost efficiency.

-Identification of supporting research requirements and provision to obtain this by in-house investment or extra-mural contract.

In addition to providing a framework for planning the detailed work program, the SP document provides a record of continuity within the company over a time span when personnel are bound to change. It should be a dynamic document - representing the best recommended practice at a particular point of time - and will require periodic updating. This update is itself a good discipline as the breeder is forced to define why some new course of action is to be preferred (there are fashions in tree breeding, as in most other areas of endeavour, and much time can be lost if breeders are overly responsive to the latest pronouncement from gurus in North America, Australasia or elsewhere!).

I have said little about the costs of investment in an FYIP. This is because each company must form its own view on the appropriate proportion of project capital to invest in this and other long-term R&D activities. An experienced breeder will be able to design a worthwhile program for a wide range of budgets. However it is worth noting the advice of Zobel & Talbert (1984) "a tree improvement program must have continuity..... Either conduct the program correctly, with total support in manpower, facilities and equipment, or do not do it at all".

This paper has perhaps discussed tree improvement as if it is a technical activity which can be carried out in vacuo. This is of course not true because of one inescapable relationship:

$$P = G + E$$

That is, the phenotype (P) (the tree we see in the plantations) is a function of both its genes (G) and its growing environment (E). A crop of good trees can fail

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through poor silviculture and the best of management will be wasted if the trees are genetically deformed, slow growing or with the wrong wood properties.

In the interests of project profitability it is therefore important for me to conclude with the suggestion that the real challenge for any technical manager is to define the balance of investment in breeding and silvicultural R&D which will maximise the value of P.

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A BREEDING STRATEGY FOR MINOR SPECIES OF EUCALYPTUS

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ABSTRACT

*The paper presents a case study of 4 generations of a low intensity tree improvement program for **E. grandis** in Florida. With some modifications, this breeding strategy may be useful for minor species (those of secondary economic importance) in Chile and elsewhere. The program has resulted in substantial realized genetic gains, while maintaining a broad genetic base. The key features of the program include: (a) a short generation time (approximately 4 years), (b) substantial infusion of new material each generation from other countries, (c) open-pollinated management of the breeding population (i.e., no control pollinations) and (d) a single field establishment that serves several functions such as selection, progeny testing and commercial seed production.*

There are various modifications of the strategy that could be made in different situations to adapt this low intensity program to a wide variety of species and program objectives. We address only a few of these possible modifications in this paper: (a) incorporating laboratory screening procedures for growth and frost resilience, and (b) using multiple populations and sublines instead of a single planting of a unified base population.

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INTRODUCTION

The forestry sector is growing rapidly in Chile with the current harvest of 13 million cubic meters expected to nearly double by the year 2010 (McNutt, 1993). Further, nearly 2/3 of this increased production is projected to be available for export, making forest products an increasingly important component of the Chilean economy (McNutt, 1993).

The total plantation estate (currently at about 1.5 million hectares) is growing at a rate of 30,000 to 50,000 ha per year. At present, over 80% of these plantations are composed of radiata pine (Jayawickrama **et al.**, 1993; McNutt, 1993), but eucalypts are also becoming a significant part of the plantation estate. By 1992 there were an estimated 170,000 ha of eucalypt plantations expected to increase to an equilibrium value of 300,000 ha by the year 2000 (Jayawickrama **et al.**, 1993). The production of eucalypts will be complementary to that of radiata pine allowing Chile to compete in some short fiber markets.

More than 75 species of eucalypts have been tested in Chile in well-designed species trials (Barros 1989 as cited by Jayawickrama **et al.**, 1993), and eight promising species have been identified for different climate/soil combinations in Chile (INFOR 1986 as cited by Jayawickrama **et al.**, 1993): **E. camaldulensis**, **E. cladocalyx**, **E. delegatensis**, **E. fastigata**, **E. globulus**, **E. nitens**, **E. regnans** and ***E. sideroxylon***.

With plantation area projected to grow to 300,000 ha in the near future, there is certainly enough economic justification to begin domestication efforts through genetic improvement, and tree improvement plans have been developed for 5 of the 8 promising species (Infante **et al.**, 1991): **E. camaldulensis**, **E. delegatensis**, **E. globulus**, **E. nitens**, and ***E. regnans***. These tree improvement plans are very well formulated and call for two lines of strategy. In the short-term program, the emphasis will be on the utilization of existing stands of eucalypts in Chile for seed production and the introduction of new provenances and families from native stands in Australia. The long-term phase includes grafting of 250 to 300 selections of each species into clone banks, followed by crossing and progeny testing.

The purpose of this paper is to describe a tree breeding strategy that may be useful for some of the species of eucalypts in Chile that are of minor economic importance or for one of the 5 major species to develop a variety for a certain locale (climate or soil type) or for a specific product. This system has been successfully

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used for 4 generations of breeding ***E. grandis*** in the southern part of Florida in the USA (Reddy and Rockwood, 1989; Franklin, 1986a; Meskimen, 1983). With modifications, the system may be appropriate for many species of minor importance or for special situations (climates, soils, products) in the breeding of major species.

The paper begins with a brief discussion of the breeding strategy currently being employed for **E. grandis** in Florida, and then presents some possible additions and modifications to the strategy that may be useful in certain situations.

BREEDING STRATEGY AND GENETIC GAINS FROM THE *E. grandis* PROGRAM IN FLORIDA

A small tree improvement effort with **E. grandis** began in south Florida in the mid-1960s. The key features of the strategy employed have been (Reddy and Rockwood, 1989; Rockwood **et al.**, 1989; Franklin, 1986b; Meskimen, 1983):

1. A short generation time (approximately 4 years) involving initial early selection at 1.5 to 2 years (which is approximately 25% of the 8-year rotation age) followed by the use of progeny test and pedigree information to guide seed collections for commercial production at ages 6 and thereafter;

2. Substantial infusion of new material (individual mother tree collections from outside Florida) to each generation's base population to increase the genetic base especially for provenances from Australia not adequately represented;

3. A single field location (containing approximately 500 open-pollinated families arranged in a completely randomized design with 60 single tree plots per family) that combines 4 functions in the following temporal sequence: (a) genetic base population, (b) a progeny test of the previous generation, (c) partial seed source of the next generation's base population, and (d) a commercial seedling seed orchard; and

4. Open-pollinated management of the breeding population that obviates the need for any control pollinations.

The exact procedures used in each generation have evolved over the four generations of the program. The details provided here are representative of the more advanced generations and emphasize the concepts of the breeding strategy (Figure 1).

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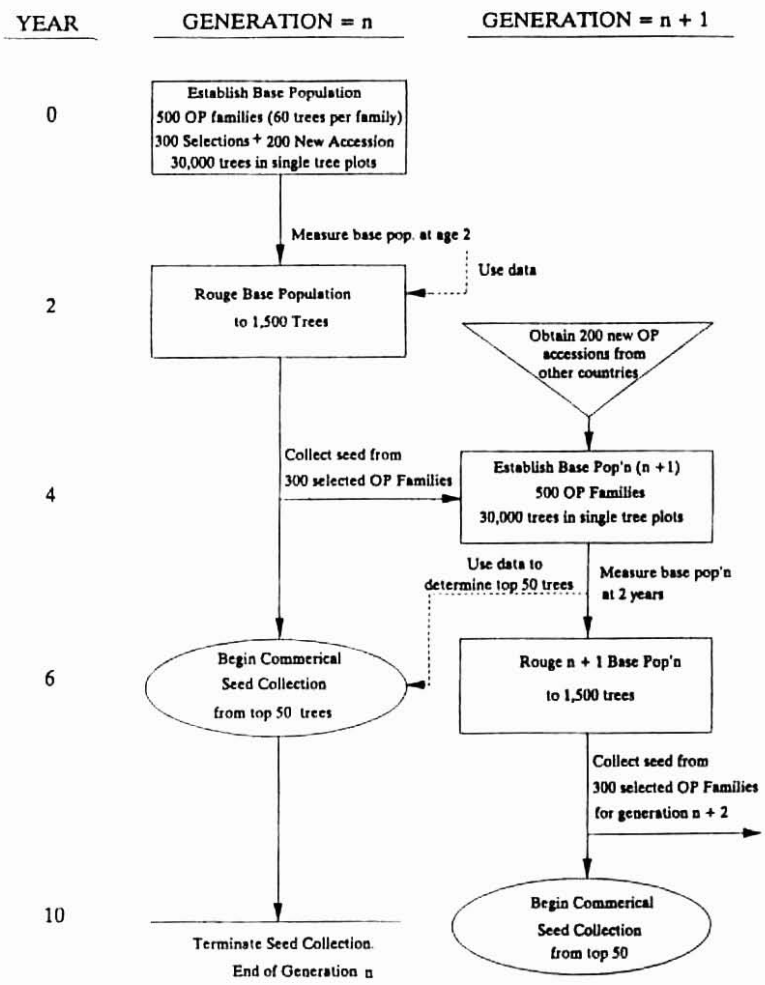

Figure 1. **SCHEMATIC REPRESENTATION OF 2 GENERATIONS (labeled n and n+1) OF THE BREEDING STRATEGY USED FOR *E. grandis* IN FLORIDA.**

Each generation, called generation number n, begins in year 0 by importation of 100 to 200 new accessions (usually open-pollinated, OP, families from individual trees in Australia or other countries). These are combined with 300 to 400 OP families selected from the previous generation to form the new base population of 500 OP families. These 500 OP families are established on a single representative site (15 ha) using a completely randomized design with 60 trees per family allocated to single tree plots. Unique features of the nursery production, tree labeling, field layout, planting procedures, monumentation and record keeping are described elsewhere (Reddy and Rockwood, 1989; Franklin, 1986b; Meskimen, 1983).

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At age 2 years (mean height of 5 m), the base population is measured for several traits (e.g., growth, frost resilience, form) and the data are used in two ways: (a) Family rankings are used to rogue the previous generation's seedling seed orchard (which would be 6 years old and on a different site location), (b) Family rankings and individual tree performance are used to thin the current base population from 30,000 to 1,500 tree (an intensity of 1 in 20, Reddy **et al.**, 1986). The selection retains 400 of the original 500 OP families with better families contributing more individuals (up to 10). To keep a broad genetic base, there is an effort to retain at least 1 tree from half of the 200 new accessions (which typically do not grow as well as the advanced-generation selections).

At age 4 years, OP seed are collected from 300 trees (out of the 1,500 present) and combined with 200 new accessions to form the next generation's (n+1) base population (which is planted on a similar site location in the exact field design as described above). Progeny test data and pedigree information are used to guide the selection of the 300 trees with the objective of maximum genetic gain subject to the constraints of minimum relatedness and a broad genetic base.

The field location now containing 1,500 trees (generation n) has one remaining function; it is a seedling seed orchard for commercial seed production. The newer field location (the n+1 generation base population described above containing 30,000 trees) is measured at age 2 years, and these progeny test data are used to identify the best 50 seed orchard trees for seed collection. All 1,500 trees are left to grow in the seed orchard to provide a broad genetic base for pollen and to minimize inbreeding, but seed are collected from only the top 50. Both family and within family information are used to ensure that these are the best 50 trees in the orchard. These same trees can be used for production of hybrids or for cloning via rooted cuttings (Meskimen **et al.**, 1987) or micropropagation (Warrag **et al.**, 1990; Meskimen **et al.**, 1987).

This completes an entire cycle of breeding and commercial seed production. At any one time there are two generations (two field locations) in place simultaneously (Figure 1). The field location for generation "n" is serving the function of commercial seed production (from ages 5 to 9), while the field location for the "n+1" generation is functioning as a base population and progeny test.

The main traits selected for in the **E. grandis** program have had moderate individual tree heritabilities: approximately 0.3 to 0.4 for both seedling and coppice height and volume growth; and 0.3 for frost resilience (Reddy and Rockwood, 1989; Franklin, 1986a). Further there have been substantial realized gains (Table 1), even while maintaining a broad genetic base. A study investigating the impact of inbreeding in the fourth-generation seed orchard indicated a very small impact of inbreeding depression on genetic gains primarily due to the large population of trees (1,500) still remaining in the orchard.

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Formally, the heritabilities and genetic gains reported apply only to material planted back on this site and may be upwardly biased if substantial genotype x environment exists. Nevertheless, the results demonstrate that a low intensity program, characterized by open-pollinated management of the breeding population and with a single field test location serving several functions, can produce substantial genetic gains in a short time period.

Table 1.

REALIZED GAINS FROM FOUR GENERATIONS OF THE E. grandis **PROGRAM IN FLORIDA. VOLUME AT 2.5 YEARS IS FOR SEEDLING GROWTH (Franklin, 1986 b), WHILE THE OTHER THREE TRAITS ARE FOR COPPICE GROWTH AFTER HARVEST (Reddy and Rockwood, 1989). NOTE THAT THE FIRST-GENERATION POPULATION WAS POORLY ADAPTED TO FLORIDA CLIMATE AND SOILS, THUS MAKING THE GENETIC GAINS OVER THE FIRST GENERATION ATYPICALLY LARGE.**

Generation	Volume at 2.5 yrs (dm^3)	Coppice Volume at 5 yrs (dm^3)	Coppice height at 5 yrs (m)	Frost Resilience at 5 yrs[a]
1	7.5	7.0[b]	5.0	1.75
2	14.6 (95)[c]	21.5 (207)	7.8 (56)	1.39 (20)
3	17.0 (127)	25.9 (270)	8.4 (68)	1.34 (23)
4	19.7 (163)	40.2 (474)	9.4 (88)	1.25 (29)

[a] Frost resilience was scored from 0 to 3 with a 0 indicating no frost damage.
[b] Trees with coppice growth experienced 2 significant freeze events not experienced by the seedlings. This accounts for the lower mean annual increment of coppice trees.
[c] Numbers in parentheses indicate the percentage genetic gain above the first generation.

POSSIBLE MODIFICATIONS TO THE FLORIDA E. grandis BREEDING STRATEGY: LABORATORY TESTING

While the strategy outlined above has been successful in producing genetic gains in each of four generations, the are several minor modifications that might adapt the strategy to a range of different species or situations. We address a few of these possible modifications that might be useful in Chile given the large range of climate, soils, product objectives and species of interest.

In the above strategy, the only genetic information available on both family and seedling performance comes from the measurement of the base population at age 2 years (Figure 1). Recently, laboratory procedures have shown promise for conducting growth and frost resilience tests on young

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seedlings (Merwin, 1993; Rockwood **et al.**, 1993; Tibbits **et al.**, 1991; Raymond **et al.**, 1986). Through such procedures, a larger number of trees can be assessed, resulting in higher selection intensities and therefore higher genetic gains. It is theoretically possible to conduct these testing procedures without extending the generation interval, thus genetic gains per unit time would also be increased. For example, two- to three-month-old seedlings could be used to develop family rankings that would allow poorer growing, less frost resilient families to be identified and not selected as part of the 300 OP families moved to the next generation's base population (Figure 1). The information could also be used in the current generation to help identify the top 50 families to use for commercial seed production, thus increasing the genetic quality of seed from the seed orchard phase.

The screening procedures for frost resilience seem particularly important because of the variability of timing, duration and severity of natural frosts from year to year. Laboratory screening methods allow a standard, repeatable procedure to be used at a specific time each generation. Freeze screening techniques using leaf disks that are frozen in laboratory cold baths have been applied to several species of ***Eucalyptus*** (Rockwood **et al.**, 1993; Raymond **et al.**, 1986), and are non-destructive since only small disks of leaf material are used. The techniques can be applied to either field-grown or greenhouse-grown seedlings; thus, screening techniques could be applied directly to the base population of 30,000 trees at age 2 when the other measurements are taken (Figure 1). This would mean that it would not even be necessary to grow additional seedlings. The screening techniques are based either on the electrical conductivity after freezing (Rockwood **et al.**, 1993; Raymond **et al.**, 1986) or microcalorimetry (Merwin, 1993). In one study with ***E. globulus*** (Tibbits **et al.**, 1991), these laboratory techniques were shown to be correlated with field results and a possible genetic gain of -1°C was estimated for typical selection intensities.

MODIFICATIONS TO THE FLORIDA *E. grandis* BREEDING STRATEGY: MULTIPLE POPULATIONS & SUBLINES

In addition to the laboratory screening techniques just described, it is also possible to make modifications of the strategy that involve different options for the structure and management of the population. Some modifications would increase the size and cost of the program dramatically (such as use of control-pollinations for management of the breeding population). We do not consider these types of major modifications, but rather describe here only two small changes in the strategy that would still permit the strategy to be useful for minor species of eucalypts. The first modification involves the use of multiple populations, while the second employs sublines.

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Both of the modifications of the strategy described below involve the use of more than one field location per generation for the management and testing of the population. We believe that these strategies described below would work very nicely in a cooperative approach of several organizations, because each organization could plant one field location per species.

While the two strategies below differ in some respects, they share one important advantage above the original strategy employed in Florida: insurance. With only one field site location as used in the original strategy, the entire program is vulnerable to a natural disaster such as fire, flood, freeze, etc. Multiple field locations separated geographically provide insurance against natural disaster.

MULTIPLE POPULATIONS: The first modification (use of multiple populations -- see below for definition) involves the use of four field locations (we chose four locations for illustration, but it could be more or less than four). In the first generation, the field locations have identical designs and family composition: the same 500 OP families with 30 seedlings of each family planted at each location in a completely randomized design or a randomized complete block design with noncontiguous plots. This would be a total of 60,000 trees planted (4 locations 500 families 30 seedlings per family). This would double the size of the program (since the original strategy employed 500 families with 60 trees per family = 30,000 total trees), but would still be suitable for a minor species if four organizations shared the effort because all seedlings are open pollinated.

The management of the field locations would proceed as described above for the original strategy: measurement at 1/4 to 1/2 of the rotation age followed by collection of OP seed from 300 trees to combine with 200 new accessions to form the new generation's base population. However, the use of multiple field locations could increase genetic gains in several ways. First, the progeny test data can be combined across sites to increase the precision of the family rankings and therefore increase the gains from family selection (which is a key element in choosing the top 50 trees at each location to collect commercial seed from each generation).

Second, it is possible to quantify the amount and type of genotype x environment interaction that exists across the 4 locations. If the 4 field locations are owned by four different cooperators with forest lands in different regions of Chile or with very different soil types, then genotype x environment interaction could be very important. It would then be possible to manage each of the 4 field locations for its own special environment. That is, the selection in the first field location could still use data from the other 3 locations, but with most of the weight (or emphasis) placed on performance at that location. Then, over a few

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generations, the four populations would begin to diverge genetically as they begin to form four land races -- one land race for each of the four different types of climate and soils.

These four different populations would be called multiple populations (Namkoong **et al.**, 1989 p 71; Burdon and Namkoong, 1983; Namkoong, 1976), because each is being bred for a different environment. Multiple populations have been used successfully in breeding programs for both major species (White **et al.**, 1992; Carson **et al.**, 1990) and minor species (Barnes and Mullin, 1989; Barnes, 1986; Barnes **et al.**, 1984). The use of multiple populations is not rigidly structured, but rather provides a large amount of flexibility in each generation and across several generations.

As an example of the flexibility of using multiple populations, suppose that one cooperator has a strong interest in developing certain fiber characteristics for a special product. That cooperator could test and select for that particular product objective in the field location located on their forest land. Another cooperator might be planning on managing operational plantations by coppicing. Then that cooperator would cut down all trees at their field location and measure the performance of the coppice re-growth. Each of the four multiple populations could be managed and bred for a different set of environments and product objectives; thus, they would diverge genetically over generations.

There are many other examples of the way that multiple populations could be used, and all permit great flexibility. As one final example, material can be exchanged between the four multiple populations at any time. Remember that the strategy requires that new infusions are added to the selected OP families from the previous generation to form the new base population of 500 families (Figure 1). In the original strategy, these new 100 to 200 OP families came from other countries, but with the use of multiple populations, a portion of the new accessions to each multiple population could be the best material from the other 3 multiple populations.

SUBLINES: The second modification of the original strategy uses sublines. Using sublines appears similar at first to using multiple populations; however, there are distinct differences. The purpose of sublines is to completely eliminate inbreeding depression in material planted in commercial plantations, and this approach requires that different sublines be completely unrelated (Burdon and Namkoong, 1983; McKeand and Beineke, 1980; van Buijtenen and Lowe, 1979; Burdon **et al.**, 1977; van Buijtenen, 1976). This reduces the need for large numbers of new infusions each generation to maintain a broad genetic base because the sublines are unrelated -- meaning that crosses between sublines will be unrelated, and therefore not suffer from inbreeding depression.

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For an example of using sublines, we assume that only two sublines are needed because either vegetative propagation or directed control pollinations between sublines will be used for production of commercial propagules (see White 1992 for a discussion of the number of sublines required). To keep the size of the program at 60,000 trees planted each generation (same size as the previous modified strategy with multiple populations), we suppose that there will be eight cooperators with each cooperator putting in one field location designed as follows. First, the 500 OP families that form the original base population in generation 1 are divided in half (250 are placed in subline I and 250 are designated for subline II) in such a way that all genetic relationships are maintained within a subline. Thus, any family in subline I is completely unrelated to every family in subline II.

Next, each of the eight field locations is established in a completely randomized or randomized complete block design (non-contiguous plots) containing 30 trees from each of 250 families from either subline I or subline II. Thus, each location has 250 families 30 trees per family = 7,500 trees (1/2 the size of each test location in the multiple population example and 1/4 the size of the 30,000-tree location in the original strategy). Of the eight locations, four of the locations contain the exact same families (subline I) and the other four contain the 250 families of subline II. Thus, the progeny test information still comes from 4 locations instead of a single location which increases genetic gain.

The field locations could still be used as seedling seed orchards for at least few generations (assuming that new accessions were infused each generation), but the inbreeding level will build up more rapidly because each location contains 250 instead of 500 families. Thus, eventually it might be necessary to use only crosses of material between sublines to generate propagules for commercial planting. This would ensure that the offspring from these crosses would not suffer inbreeding depression because material in subline I is unrelated to that in subline II. This need to rely mainly on vegetative propagation would probably not happen for 5 to 8 generations, and could be delayed by reducing selection pressure or by infusing of more new accessions each generation.

Two aspects are critical to maintaining the integrity (unrelatedness) of the two sublines across the generations: (1) The material infused into subline I each generation from new sources must be genetically unrelated to the material being infused into and the material already contained in subline II (and vice versa), and (2) No exchange of material is permitted between sublines.

Within each subline, the four field locations can be considered as identical replications of each other, or they can be managed as four different multiple populations exactly as described above for the multiple population strategy. With the latter scenario, the eight field locations might be divided into 4 pairs. Each pair (1 test location of subline I and one test location of subline II) would be bred for

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the exact same product objectives and the same climate and soil characteristics -- this is the concept of multiple populations and there would be 4 multiple populations. However, the two locations within each pair would start off with 250 different and unrelated families and would always remain unrelated -- this is the concept of sublines and there are always 2 sublines. Material could be exchanged each generation between the 4 multiple populations within a subline, but not between populations that are in different sublines. This combined subline/multiple population approach dramatically increases the potential for genetic gain because there are two populations being bred for the same exact set of selection objectives and these will always be unrelated.

SUMMARY AND CONCLUSIONS

The simple, low intensity breeding strategy used for ***E. grandis*** in Florida has employed a single field location each generation and been based on: (1) open-pollinated management of the breeding population, (2) high selection intensity, (3) rapid turnover of generations and (4) substantial infusion of new material from other countries in each generation to maintain a broad genetic base.

This strategy, which has been successful in achieving substantial genetic gains for a relatively low cost, can be modified in many ways to make it useful for a variety of tree species of minor economic importance. We discuss two such modifications in this paper (multiple populations and sublines) that we believe would both enhance genetic gains and lend themselves to a cooperative approach with each cooperator only establishing a single field location of 7,500 or 15,000 trees (3.75 or 7 ha) each generation.

There are many other possible modifications, and even with modification, this type of strategy is not suited to all situations. Each breeding strategy needs to be developed and tailored to a specific situation and the exact strategy best for any situation depends on the biological and characteristics of the species, the product objectives, the diversity of climate and soils and the logistical capabilities of the organization or cooperating organizations.

Nevertheless, we believe than the basic strategy presented here is useful for minor species and might be useful for some eucalypt species in Chile. The large amount of infusion in the early generations allows for new provenance and family material to be tested from other countries or from selections made in Chilean land races. In later generations, the number of infusions might decrease as its genetic quality falls below that of the advanced-generation Chilean material.

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Finally, the open-pollinated management of the breeding population is a key feature of this strategy which permits many more trees to be grown each generation because the seed are so much easier to obtain. This allows a high selection intensity to be used, maintains a broad genetic base and greatly reduces the cost and time of breeding. Open-pollinated or polymix management of breeding populations has been suggested for other programs (White **et al.,** 1992; Cotterill, 1989;Cotterill **et al.**, 1989), and is especially well suited to the improvement of minor species. The loss of the paternal side of the pedigree and hence the potential for build up of unknown amounts of relatedness in the breeding population is the major disadvantage. By taking steps to alleviate this problem (such as use of sublines and/or the infusion of new material), the build up of relatedness will not be a problem that will lead to appreciable inbreeding depression of the seedlings or plantlets commercially deployed.

In conclusion, there may be opportunities in Chile and elsewhere to use a simple, low cost breeding strategy such as that employed for ***E. grandis*** in Florida for the improvement of tree species of minor economic importance or for single companies or organizations that must carry the full load of breeding for a given species. This would allow improvement of a wider range and larger number of tree species than might otherwise warrant a genetic improvement program.

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INFOR

GENETIC MODIFICATION OF EUCALYPTS - OBJECTIVES, STRATEGIES AND PROGRESS

John G. Purse (*)

ABSTRACT

Genetic modifications (GM) offer opportunities to introduce traits that are not present, or to modify those that are difficult to breed for, in the commercially-important eucalypts. In order to express the new or modified traits at a consistent, desired level in eucalypts (or any heterozygous species), we believe that commercial applications will be restricted to situations in wich operational clonal propagation is possible. In order to introduce the new/modified traits quickly, it will be necessary to introduce them directly into operational clones. For any particular clone/trait combination, a number of modified lines will need to be produced, so that lines expressing the introduced gene(s) at the appropriate level can be selected for operational use.

The Shell Research programme aims to develop the technology to introduce genes of commercial importance into operational clones used by Shell Group Forestry Companies. Our intention is to use this technology to introduce genes developed by other organisations. Our current effort focusses on using of interest to us are lignin modification/reduction, and cold tolerance of unhardened trees.

During 1993 we have successfully conducted a field trial on genetically-modified ***E. grandis*** *containing a marker gene, at Sittingbourne. This trial received approval from the UK authorities that regulate experimental release of genetically-modified organisms into the environment. We expect to screen* ***E. grandis*** *clones containing the glyphosate-tolerance genes in our greenhouses from late 1994. For* ***E. globulus****, application of GM technology requires from development of an efficient system for shoot regeneration from clonal tissues in vitro, and the identification of candidate operational clones that can be propagated by cuttings on an operational scale.*

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INTRODUCTION

In the past 12 years, many companies and organisations have invested substantial effort and resources in developing genetically-modified crop plants. Such plants contain genes for traits not present in the original genepool, or for traits that are difficult to breed for. Scientifically and technically these efforts have been successful for many crop species and genes. The first products of the new technology are now about to reach the market.

The interest in applying this technology to tree species is widespread, though progress has been slower than with the major arable crops. This slower progress reflects both some technical difficulties of genetic modification (GM) in many woody species compared to annual crops, and fewer situations in which the potential benefits appear to justify the development costs.

The short rotations on which plantation eucalypts are frequently grown (7-15 years) makes them one of the most obvious candidates for application of GM technology in plantation forestry. At the Shell Research laboratories in Sittingbourne, we are developing GM eucalypts for use by Shell Group Forestry companies. The purpose of this paper is to discuss the overall objectives of this programme, and the strategic issues that have governed its development. The paper also contains a summary of our progress to date. The objective is not to present detailed technical information, but to discuss issues that will be relevant to any organisation intending to develop and/or use GM technology for plantation species.

PROGRAMME OBJECTIVES

At present, a requirement for modification or introduction of any trait by GM is that the trait concerned is regulated by the activity of only one or two characterised genes. (This does not necessarily mean that traits under polygenic control are not susceptible to GM, but in these cases it is still necessary to identify and characterise key genes whose regulation is likely to influence expression of the trait to an economically significant level). This also implies that considerable knowledge exists on the biochemistry and physiology underlying expression of the trait.

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We have identified three traits that we believe meet these scientific and economic criteria in plantation eucalypts. The objective of our R&D programme is to evaluate the practical and economic consequences of introducing or modifying these traits.

Herbicide Tolerance

It is well-known that eucalypts are particularly sensitive to weed competition prior to canopy closure. A range of silvicultural approaches to post-planting weed control are available, based on cultivation and/or selective herbicides. The availability of eucalypts tolerant of a non-selective herbicide would permit more cost-effective weed control to be practised. Of the non-selective herbicides, the environmentally benign characteristics of glyphosate make it attractive for such purposes. Genes for glyphosate tolerance have been developed by Monsanto, and we have licensed these genes for evaluation in eucalypts.

Frost Tolerance

The incidence of frosts is a major factor determining the locations in which eucalypts can be grown successfully. Certain of the more temperate species of eucalyptus are tolerant of temperatures as low as -15°C, provided they have been suitably hardened. Considerable genetic variation in frost tolerance in the hardened state exists, and can be exploited by conventional breeding and selection (Potts and Tibbits, 1991; Tibbits, 1991; Raymond **et al.**, 1991). By contrast, little variation in frost tolerance appears to be present in unhardened eucalypts, and such plants are usually killed at around -4°C. Thus, eucalypts are particularly vulnerable to sudden, unseasonal frosts, and there appears to be little scope to improve this by conventional means.

Recent advances in our understanding of frost-tolerance mechanisms in plants, and in particular the role of 'anti-freeze proteins' that occur naturally in hardened plants (Griffith **et al.**, 1992), suggest that expression of these proteins in unhardened shoot tissue, using a GM approach, would confer extra frost tolerance. Evidence from other species (DNA moleculas improving cold, resistance, 1990) suggests that this approach is realistic.

We have started a project to evaluate the validity of this approach in eucalyptus.

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Lignin Reduction and/or Modifications.

There are substantial costs associated with removing lignin during chemical pulping, and of bleaching the resulting pulp to remove residual lignin.

Cost reductions through changes to the lignin content and/or composition of the wood feedstock are potentially attractive. The scope to do this by conventional breeding appears limited, due to the narrow variation of lignin content within eucalyptus species.

Knowledge of the controls on the pathways of lignin biosynthesis is advancing rapidly, and a range of enzymes are candidates for regulation of lignin biosynthesis. The consequences of reducing the activity of any one of these enzymes, on lignin content or other growth characteristics, in trees is unclear. It appears prudent to seek only small or modest changes in total lignin, but we believe that such changes would be significant economically. We are currently studying options for evaluating GM technology for lignin modification in eucalypts.

PROGRAMME STRATEGIES

It is evident from the above comments that GM is not an alternative to conventional tree improvement programmes. GM cannot possibly achieve the progress offered by conventional breeding. The potential of GM lies in the scope it provides to introduce genes for traits that are either not present in the genepool of a breeding population, or for which there is little or no variation.

Thus, GM is an approach that offers scope to enhance the value of tree improvement programmes, provided it is an integral part of a tree improvement strategy. Aggressive eucalyptus improvement programmes can have intervals between generations as short as five or six years. Such programmes will deliver new sets of genotypes for operational use regularly at such intervals. In these situations, the commercial benefits of GM will be realised most rapidly. The challenge for biotechnologists will be to keep pace with the breeders, otherwise new GM genotypes may quickly be superseded by more valuable non-GM genotypes from the next generation.

A further determinant of strategy is that eucalypts, in common with most woody species, are heterozygous. Essentially, every seed produced in a seed orchard, or even in a full-sib family, will be genetically unique. The value of GM is that traits can be expressed at a precise level, but this cannot be expected to occur in progenies if GM parents are used in a seed orchard. A bag of

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eucalyptus seed in which only 50% of the seeds have a useful level of glyphosate tolerance will not have much added value if used operationally! The obvious conclusion is that commercialisation of GM technology in eucalyptus and other tree genera can only be achieved through clonal propagation. Application of the technology will be most rapid and efficient if candidate operational clones are selected for GM. In other words, tested clones of known value would be 'fine-tuned' by insertion of new genes through GM. In the longer term, it is likely that breeders will modify their strategies in order to incorporate GM traits into breeding populations.

These considerations have guided the approach we have taken in developing the technology for GM of eucalypts. Consequent key aspects of our approach are:

1. The technology will be applied only to operational, or candidate operational clones. Propagation for operational use is likely to be by cuttings; such clones will have been selected for acceptable levels of rooting.

2. Adoption of the technology ideally requires that any clone selected by a forestry company can have the desired genes inserted by GM. Thus, the techniques involved should be applicable as far as possible to all genotypes of all target taxa. This will avoid an additional non-yield related selection criteria being imposed on clones.

The steps involved in our GM process are:

1) Establishing clones in our greenhouses at Sittingbourne.

2) Establishing micropropagated cultures of these clones.

3) Introducing the gene(s) of interest into tissue cultures derived from the micropropagated cultures, using a system based on the bacterium ***Agrobacterium tumefaciens.*** One of the genes has to permit the modified cells to be selected and grown on following this step, while non-modified cells do not develop further; such genes are called selectable marker genes.

4) Growing cultures of the modified cells, and regenerating shoots from these *in vitro*.

5) Multiplying each of a set of regenerating shoots of each GM clone, using micropropagation, to produce a set of GM lines derived from each clone. Rooting the shoots produced to give ramets of each line.

6) Screening the resulting plants to determine the lines that express the

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introduced genes at the desired level. This will be carried out initially in the greenhouses at Sittingbourne. Further evaluation will need to be carried out in locations where the GM clones subsequently will be used operationally.

RESEARCH PROGRESS TO DATE

Plant Material

We developed the basic greenhouse techniques for importing, propagating and exporting eucalyptus clones prior to starting our GM programme. We have continued to refine these techniques, and we anticipate that they already meet the requirements of our GM programme, for all species and hybrids of commercial importance.

Micropropagation

In common with other organisations, we have developed micropropagation procedures for eucalyptus clones. The species we have studied are ***E. grandis***, ***E. globulus*** and ***E. nitens***. In addition we have studied clones of hybrids of ***E. grandis*** with ***E. camaldulensis, E. tereticornis, E. urophylla*** and ***E. nitens***. Almost all clones can be multiplied *in vitro*, and all clones that can be multiplied can be rooted in vitro. (The clones we have studied are ones that have already been selected for acceptable levels of rooting as cuttings, so it is unsurprising that they root *in vitro* also).

Genetic Modification of Tissues

We have designed a gene delivery (vector) system that is compatible with all ***A. tumefaciens*** strains, and which delivers only the target DNA to the plant genome. We have used our vector to identify a strain of **Agrobacterium** that is highly effective in eucalypts. We have used this strain/vector combination to insert genes into eucalyptus cultures. The vector contained a marker gene (a β-glucuronidase gene, GUS) and a selectable marker gene (kanamycin resistance). Under selection on a medium containing a kanamycin analogue, at least 20% of explants produce GM callus. All species and clones we have studied respond in a similar manner. This is acceptable for practical purposes.

Shoot Regeneration

Cultured explants of eucalyptus seedlings can be induced to form shoot

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meristems *de novo* on appropriate media (Warrag **et al.,** 1992; Subbaiah and Minocha, 1990). We have extended this to explants derived from ***E. grandis*** clones (Laine and David, 1993), to other species (including ***E. globulus***), and to hybrids also. At present, the frequency of regeneration is only sufficient for practical use in certain clones. However, scope to extend the range of clones that respond well appears to be present, because many clones show some regeneration. This aspect is a major focus of our work at present.

Production of GM Plants

We have integrated these techniques to generate GM-lines of ***E. grandis***. Three of the first lines, generated from seedlings in 1992, were used in a field trial conducted at Sittingbourne between June and October 1993. The GM lines contained GUS and kanamycin resistance genes; isogenic non-GM lines and seedlings acted as controls. The objective of the trial was to determine whether the genes were expressed uniformly in the plants, and whether the plants grew normally. As far as we are aware, this was the first field trial of GM eucalypts in the world.

Because the planned trial involved release of a genetically-modified organism, we had to obtain approval from the UK government authorities for the trial. We received this approval and experienced no difficulties in conducting the trial.

As anticipated, the GM plants grew normally, and the introduced genes were expressed uniformly within the ramets of each line. Thus, we believe that we are now well placed to conduct field trials of clones containing potentially valuable genes. We are intending to conduct such trials from 1995 onwards.

The possibilities available to the Forest industry through application of this technology clearly are exciting. However, it is worth noting that many aspects of the gene technologies involved are covered by patents, or are the subject of patent applications, from biotechnology companies. Our experience is that considerable effort is required to keep abreast of patent issues, and that it will be necessary to pay licence fees to use proprietary technology developed by others.

ACKNOWLEDGMENTS

Progress on this programme has been possible only with the inputs of many people in the team at Sittingbourne, notably Sally Brown, Glyn Edwards, Neil Fish, Keith Fuell, Michael Keil, Trevor Wignall and Gary Williamson.

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RESULTADOS DE PRIMERAS TEMPORADAS DE HIBRIDACION EN Eucalyptus spp.

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RESUMEN

Se dan a conocer resultados de las primeras temporadas de polinizaciones controladas, para la obtención de híbridos de interés para el programa FYIP (Fiber Yield Improvement Programme) de Santa Fe.

La hibridación ofrece el potencial de combinar características deseables entre especies como: crecimiento, enraizamiento, propiedades de la madera (densidad básica y otras características pulpables) y resistencia a condiciones de estrés (sequía o heladas). Las especies involucradas en este programa son: ***E. globulus (EGG), E. maidenii (EM), E. nitens (EN), E. camaldulensis (EC), E. bicostata (EB) y E. viminalis (EV).***

Para la primera temporada (1991/92), fueron experimentados 6 tipos de cruzamientos controlados totalizando 1.622 flores emasculadas. Los mejores resultados fueron con ***ENxEGG y ENxEC,*** *con 34 y 43% de prendimiento de cápsulas y un promedio de 5,6 y 5,5 semillas por cápsula, respectivamente. Para la segunda temporada (1992/93) se experimentaron 5 cruzamientos y se efectuaron 3084 emasculaciones. Los resultados de los cruzamientos fueron:* ***EGGxEGG*** *con 62% de prendimiento,* ***ENxEN*** *con 58%,* ***ENxEGG*** *con 43%,* ***EVxEGG*** *con 50% y* ***EVxEV*** *con 49%.*

Se describen las técnicas y metodologías empleadas. Se efectuó un análisis de varianza para verificar diferencias significativas entre fuentes de polen en matrices de ***EN.*** *Se estableció la relación semilla/cápsula para polinización abierta y polinización controlada en el cruzamiento de* ***ENxEGG.***

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INTRODUCCION

La hibridación artificial en especies del género **Eucalyptus** ya data de por lo menos dos décadas (Martin, 1987). La mayoría de estos programas trabajan con híbridos de **Eucalyptus spp** de climas tropicales y subtropicales los cuales son propagados clonalmente por estaquillas. La silvicultura clonal de estas especies resulta muy atractiva en términos económicos, por la homogeneidad de los bosques y de la madera obtenida para pulpa.

Las especies de interés para el programa de hibridación de Forestal y Agrícola Monteaguila S.A. se agrupan en el subgénero Symphyomyrtus tales como **E. globulus, E. nitens, E. viminalis, E. grandis, E. bicostata, E. maidenii y E. camaldulensis.**

El objetivo del programa es obtener híbridos que se adecuen a las condiciones edafoclimáticas del patrimonio de Forestal y Agrícola Monteaguila S.A. y cumplan con los requerimientos pulpables Kraft de la planta industrial de Santa Fe.

Un ejemplo es el F1 de **ENxEGG,** el cual reuniría las características de crecimiento y resistencia al frío de **EN** y la calidad pulpable de **EGG** (densidad básica y rendimiento pulpable).

Estas plantas híbridas presentan patrones morfológicos intermedios entre las especies puras (Tibbits, 1988) y los resultados de tecnología de la madera son bastante promisorios (Tibbits com. pers.). Estas plantas híbridas pueden expresar el vigor híbrido "heterosis" o presentar características anormales "runts".

De acuerdo a Griffin (com. pers.), el éxito en la generación de híbridos, tanto intraespecíficos como interespecíficos, es el conocimiento detallado de la biología reproductiva de las especies involucradas en el programa.

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OBJETIVOS

- Obtener semilla híbrida con las especies de interés para la empresa.

- Comparar número de semillas por cápsulas entre polinización abierta y controlada.

- Entregar antecedentes básicos de la fenología de las especies **E. globulus** y **E. nitens.**

MATERIAL Y METODO

Monitoreo de Fenología

Debido a que la colecta de polen y las polinizaciones controladas están condicionadas por la floración de las especies, se realizó en forma preliminar un estudio fenológico de **EN y EGG.** Para esto se seleccionaron árboles en plantaciones jóvenes menores a 5 años, representativas del patrimonio de Forestal y Agrícola Monteáguila S.A.

La Figura Nº 1 muestra la floración de ambas especies en distintos sitios de Forestal Monteáguila.

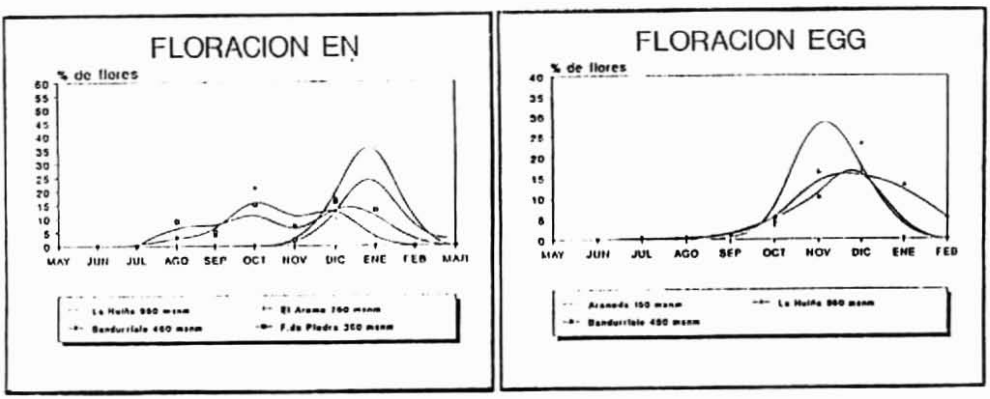

Figura Nº 1 **FLORACIÓN DE EN Y EGG TEMPORADA 91/92**

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Selección de Matrices

En la primera temporada (1991/92), los árboles madres (matrices) fueron seleccionados por la cantidad de yemas florales presentes y cuyas ramas estuvieran a una altura accesible para el trabajo en terreno (< 3 metros).

Para la segunda temporada el criterio de selección se orientó a los mejores árboles de los ensayos de introducción de especies/procedencias de la Hacienda Rucamanqui. La selección se concentró en características fenotípicas de los árboles: altura, diámetro, forma y presencia de yemas.

Selección de Polen

La colecta de polen para las dos temporadas estuvo centrada en 20 fenotipos superiores de tres Areas Productoras de Semilla (APS) de **EGG.** La colecta de polen de **EN** y **EV** se concentró en los ensayos de introducción de especies y procedencias de Rucamanqui.

De acuerdo al test de germinación de polen efectuado en laboratorio, se dejaron finalmente 10 árboles que forman un polen "mix".

Procesamiento, Almacenamiento y Germinación de Polen

El procedimiento de colecta de las yemas florales está dado por el estadio de antesis, el cual se caracteriza por la caída del opérculo.

Las ramas son sometidas al corte de sus hojas (50%) con el fin de evitar la evapotranspiración, luego son cubiertas con una bolsa de polietileno e identificadas. Posteriormente las ramas son colocadas en un "cooler" tomando la precaución que las bases estén en contacto con agua.

En laboratorio las ramas nuevamente son acondicionadas. Las bases son cortadas ya que presentan los tejidos basales oxidados, debido a la gran cantidad de compuestos polifenólicos que son muy comunes en los eucaliptos.

De esta forma el material mantiene vigor y por ende permite que el proceso de floración continué normalmente. El sistema antes descrito es conocido como colecta indirecta (de Sousa, 1988).

La etapa posterior es la colecta del polen, utilizando una espátula fina la cual retira las anteras del filamento (estambres). El material es depositado sobre

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papel aluminio en una cápsula Petri, el cual es introducido en desecadores provistos con sílica gel por 4 a 5 días. La finalidad es secar bien las anteras permitiendo el desprendimiento del polen de las tecas.

Al cumplir la etapa del secado las anteras son pasadas por una malla fina (500 µm para **EGG** y 750 µm para **EN**) con la ayuda de una brocha fina. El polen obtenido es almacenado en cápsulas de gelatina "0", las cuales son almacenadas en frascos son sílica a 4 °C. Si el polen debe ser usado en la próxima temporada este es almacenado a -20 °C.

Las muestras de polen son analizadas mediante el conteo de los granos de polen que presenten tubo polínico en una solución líquida de 150 mg/L de ácido bórico y 30 g/L de sacarosa (Potts, 1993; Griffin **et al.,** 1982), posterior a 48 horas de incubación a una temperatura de 28 °C.

Polinización Controlada

El proceso de polinización controlada envuelve dos etapas: a) Emasculación o extracción de las estructuras masculinas (anteras y estambres) y b) Polinización propiamente tal (transferir polen a la estructura femenina por un método artificial).

Las técnicas empleadas por el programa FYIP son una variante de la empleada por CSIRO. El primer trabajo en la matriz, es la selección de ramas con una buena cantidad de yemas en antesis, en el caso de **EGG** 6 a 10 yemas y **EN** aproximadamente 25 yemas. La rama es acondicionada con los mismos procedimientos de la colecta de yemas, paso posterior es la emasculación mediante bisturí o tijeras.

En seguida la rama es aislada al ser cubierta con una bolsa de polyester (Terylane).

La polinización es llevada a cabo solamente cuando el estigma está en el momento de mayor receptividad, lo cual está influenciado por la temperatura y humedad (Shivana y Johry, 1989). Para **EGG** se ha registrado que el estigma esta receptivo del 5º al 8º día y en **EN** del 7º al 10º día.

La aplicación de polen es realizada con un palito de fósforo. Concluida esta etapa, la rama es aislada con la bolsa por un período de dos semanas o más. Se cosechan las cápsulas entre el octavo y décimo mes posterior a la polinización.

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RESULTADOS

Los resultados de las dos temporadas de cruzamiento son resumidos en el Cuadro Nº 1.

Cuadro Nº 1

CRUZAMIENTOS EFECTUADOS POR PROGRAMA DE HIBRIDACIÓN. FORESTAL Y AGRICOLA MONTEAGUILA S.A.

	Temporada 1991/92				Temporada 1992/93			
Cruzamiento	Madres	Emasc.	Capsulas	Prend(%)	Madres	Emasc.	Capsulas	Prend(%)
EGG X EGG	–	–	–	–	2	89	55	61.8
EN X EGG	5	690	244	34.2	5	2012	870	43.2
EN X EN	–	–	–	–	5	605	353	58.3
EN X EC	1	179	78	43.5	–	–	–	–
EV X EGG	8	501	12	2.4	5	630	327	52
EV X EV	–	–	–	–	5	246	122	49.6
EV X EC	1	85	2	2.4	–	–	–	–
EM X EGG	1	81	17	20	–	–	–	–
EM X EC	1	86	15	17.4	–	–	–	–
EB X EGG	–	–	–	–	2	132	35	26.5
TOTAL		1622	368			3084	1762	

Se efectuó un análisis de varianza para comparar el prendimiento de cápsulas (%) en función de las matrices (**E. nitens,** temporada 1992 / 1993) y la fuente de polen (polen mix. de **E. nitens** y polen mix de **E. globulus).**

No se encontraron diferencias significativas (P> 0.05) para ninguno de los análisis desarrollados (Cuadro Nº 2).

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Cuadro Nº 2

ANALISIS DE VARIANZA EN BASE A PRENDIMIENTO DE CAPSULAS PARA MATRICES (A) Y FUENTE DE POLEN (B).

ANALISIS DE VARIANZA DEL PRENDIMIENTO DE CAPSULA PARA MATRICES (A)					
FUENTES DE VARIACION	SUMA CUADRADOS	g.l.	CUADRADOS MEDIOS	F.	NIVEL SIGNIF.
ENTRE GRUPOS	1960.6000	4	490.150000	1.797	.2667
DENTRO GRUPOS	1363.5000	5	272.70000		
TOTAL	3324.1000	9			
ANALISIS DE VARIANZA DEL PRENDIMIENTO DE CAPSULA PARA FUENTE DE POLEN (B)					
FUENTES DE VARIACION	SUMA CUADRADOS	g.l.	CUADRADOS MEDIOS	F.	NIVEL SIGNIF.
ENTRE GRUPOS	324.9000	1	324.90000	.867	.3888
DENTRO GRUPOS	2999.2000	8	374.90000		
TOTAL	3324.10000	9			

DISCUSION

Tomando como referencia el cruzamiento de **EN x EGG** temporada 91/92 (Figura Nº 2), se puede apreciar que el número de semillas provenientes de polinización controlada (PC), registra un promedio de 5 semillas versus 1 semilla de polinización abierta. Sin embargo, la cantidad de plantas germinadas de PC en vivero es baja comparada a la semilla de PA (Figura Nº 3).

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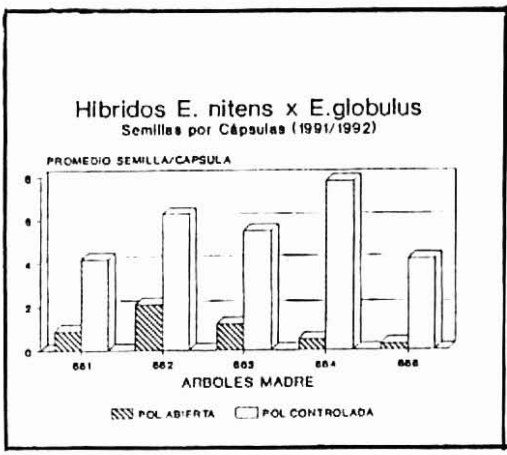
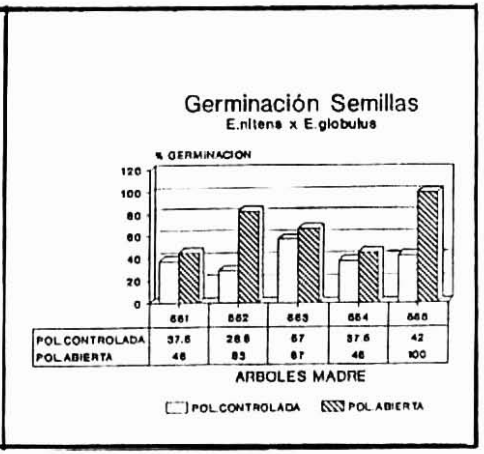


Figura Nº 2
HÍBRIDOS E. nitens x E. globulus
SEMILLAS POR CÁPSULA (1991 / 92)



	661	662	663	664	666
POL.CONTROLADA	37.6	28.8	67	37.6	42
POL.ABIERTA	46	83	87	46	100

Figura Nº 3
HÍBRIDOS E. nitens x E. globulus
GERMINACION SEMILLAS

La baja cantidad de semilla de PA puede explicarse por:
a) Menor número de vectores de polinización.
b) Arboles de la población presentan una floración asincrónica.
c) Bajo número de árboles con flores.

Si bien los resultados no muestran diferencias estadísticas de prendimiento de cápsulas entre híbridos inter e intraespecíficos, se puede apreciar en la Figura Nº 4 que los mejores resultados se obtienen en cruzamientos de la misma especie. Este hecho obedece al grado de divergencia genético/taxonómico que existe entre los individuos (Potts **et al.**,1992).

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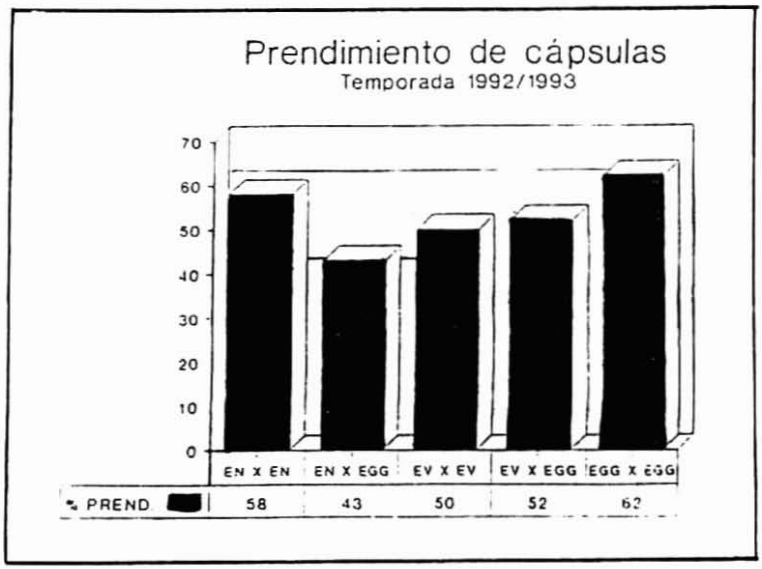

Figura Nº 4 **PRENDIMIENTO DE CÁPSULAS TEMPORADA (1992 / 93)**

El bajo número de plantas obtenidas en vivero de PC es frecuente (Tibbits, 1988). Estas plantas anómalas (runts) se caracterizan por sus hojas y entrenudos muy pequeños. Según Potts **et al.**(1992), estas plantas al cabo de un año llegan a terreno con un alto porcentaje de anormalidades e incluso se produce la muerte de las progenies.

CONCLUSIONES

Las técnicas y procedimientos empleados por el programa de hibridación han sido satisfactorios en base al prendimiento de cápsulas y producción de plantas híbridas.

Los cruzamientos controlados permiten aumentar el número de semillas por cápsula en relación a polinización abierta.

La progenie de híbridos por polinización controlada es heterogénea, ya que hay un alto porcentaje de plantas anómalas. De acuerdo a esto, el vigor híbrido o heterosis sólo se manifiesta en algunos individuos.

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RESULTADOS DE ENSAYOS DE PROCEDENCIAS Y PROGENIES DE Eucalyptus globulus EN LA ZONA CENTRO-SUR DE CHILE.

José A. Prado D. (*).
Carlos Alvear S. (**).

RESUMEN

*Se analiza el comportamiento de 230 familias de **Eucalyptus globulus ssp. globulus**, que cubren casi la totalidad del área de dispersión natural de la especie, en 3 localidades situadas entre la VII y X regiones del país.*

Las características de crecimiento evaluadas a los 4 años fueron: altura total, diámetro y volumen.

En el ensayo establecido más al norte (Cauquenes) las mejores procedencias corresponden a Otway (Victoria) y Geeveston-Dover (Tasmania). Más al sur, en la provincia de Arauco, a estas dos se agrega la procedencia del centro de la isla Flinders. Finalmente, en el ensayo establecido en Valdivia, las mejores procedencias corresponden a las islas King y Cape Barren, del estrecho de Bass, las poblaciones del oeste de Tasmania, Little Henty River, seguidas de Geeveston-Dover y Flinders Is.

Se estimaron las heredabilidades individuales para las características de crecimiento, resultando en general valores medianos a altos, sugiriendo, por lo tanto, ganancias considerables en base a la selección individual.

También se estimaron las correlaciones genéticas y fenotípicas entre las variables de crecimiento estudiadas, encontrándose valores altos.

Los ensayos establecidos constituyen una buena base para un programa de mejoramiento genético con esta especie en Chile.

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INTRODUCCION.

El **Eucalyptus globulus ssp. globulus** es una de las especies forestales de mayor importancia económica en Chile, por su rápido crecimiento, su adaptación a un amplio rango de condiciones de clima y suelo y por la alta demanda de su madera, tanto en el país como en el mercado externo.

La clave del negocio forestal en Chile está en la mantención de la competitividad en los mercados mundiales y sin duda que el aumento de la productividad de nuestros bosques es una de las vias más seguras para lograr este objetivo. Es por esto que el Instituto Forestal inició hace algunos años un programa de mejoramiento genético de la especie **Eucalyptus globulus ssp. globulus** (Prado, 1988).

Como primera etapa de este programa se decidió ampliar la base genética, mediante la importación de una colección de familias que cubre toda el área de distribución natural de la especie: el sur del Estado de Victoria; las islas del Estrecho de Bass y Tasmania.

A pesar de que esta especie es la de mayor importancia comercial entre los eucaliptos, no se disponía en el país de información sobre el desarrollo de las distintas procedencias ni menos sobre parámetros genéticos. Esto es bastante coincidente con lo sucedido en otros paises donde se planta **E. globulus**. Volker **et al.** (1990), comentan sobre la completa falta de información sobre parámetros genéticos para esta especie. Sin embargo, desde esa fecha hasta hoy día se ha publicado gran cantidad de información referente al desarrollo de procedencias y familias, incluyendo el cálculo de parámetros genéticos (Borralho **et al.**, 1992a; Borralho **et al.**, 1992b; Woolaston **et al.**, 1991).

En consecuencia, el presente trabajo constituye la primera aproximación hacia la selección genética de **E. globulus ssp. globulus** en Chile, considerando una base que cubre toda el área de su distribución natural. Esto puede conducir a una importante ganancia genética, aun cuando esta especie, a pesar de tener una distribución relativamente amplia, no presenta una gran variabilidad.

Este análisis considera solo variables de crecimiento, pero a futuro será necesario integrar otras variables que respondan a los distintos objetivos que se plantee el programa de mejoramiento. La densidad y contenido de celulosa por unidad

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de volumen son características fundamentales cuando se mejora para la producción de pulpa. La poda natural, inserción y grosor de ramas, viraje de las fibras serán de importancia para la producción de madera aserrada o chapas (Borralho **et al.**, 1992b; Whiteman **et al.**, 1992).

La otra línea de trabajo estará dada por la necesidad de superar condiciones adversas impuestas por el medio, sean estas físicas o biológicas. El mejoramiento genético de la especie podría conducir a la obtención individuos más resistentes a las heladas, sequías, plagas o enfermedades.

Las posibilidades de ganancia están, en gran parte, supeditadas a la amplitud de la base genética. El número de procedencias y familias probadas en los ensayos que se analizan en este trabajo son más que suficientes como base para un programa de mejoramiento genético.

MATERIAL Y METODO.

Antecedentes de la colección de semillas

El **Eucalyptus globulus ssp. globulus** es originario de los Estados de Victoria (VIC) y Tasmania (TAS), incluyendo las islas del Estrecho de Bass. Se le encuentra formando bosques mixtos con varias especies, entre las que se pueden mencionar **Eucalyptus viminalis, Eucalyptus obliqua, Eucalyptus delegatensis** y **Eucalyptus regnans** (Boland **et al.**, 1984). El área de distribución natural de la especie se presenta en la Figura Nº1, donde también se señalan los puntos de recolección de la semilla empleada en los ensayos de progenie.

La colección comprende 35 procedencias, representadas por 218 familias, que cubren parte importante del área de distribución natural de la especie. Además se incorporan a los ensayos dos procedencias locales (Talca y Cañete); una muestra de semilla de huerto semillero (APPM, Australia) y procedencias de otras especies estrechamente relacionadas a **E. globulus ssp. globulus,** como son **Eucalyptus globulus ssp. bicostata, E. globulus ssp. maidenii** y **E. globulus ssp. pseudoglobulus.**

El detalle del origen de estas procedencias se presenta en el Cuadro Nº 1

La colección de semilla fue realizada por CSIRO en los años 1987 y 1988, en lo que se denominó "1988 Seed Collections of **Eucalyptus globulus subsp. globulus** Labill. for Tree Improvement Purposes" (Gardiner y Crawford, 1988). Entre los criterios de selección de los árboles se encuentran: tamaño y vigor del árbol, forma del fuste, tipo de copa y tipo de ramas.

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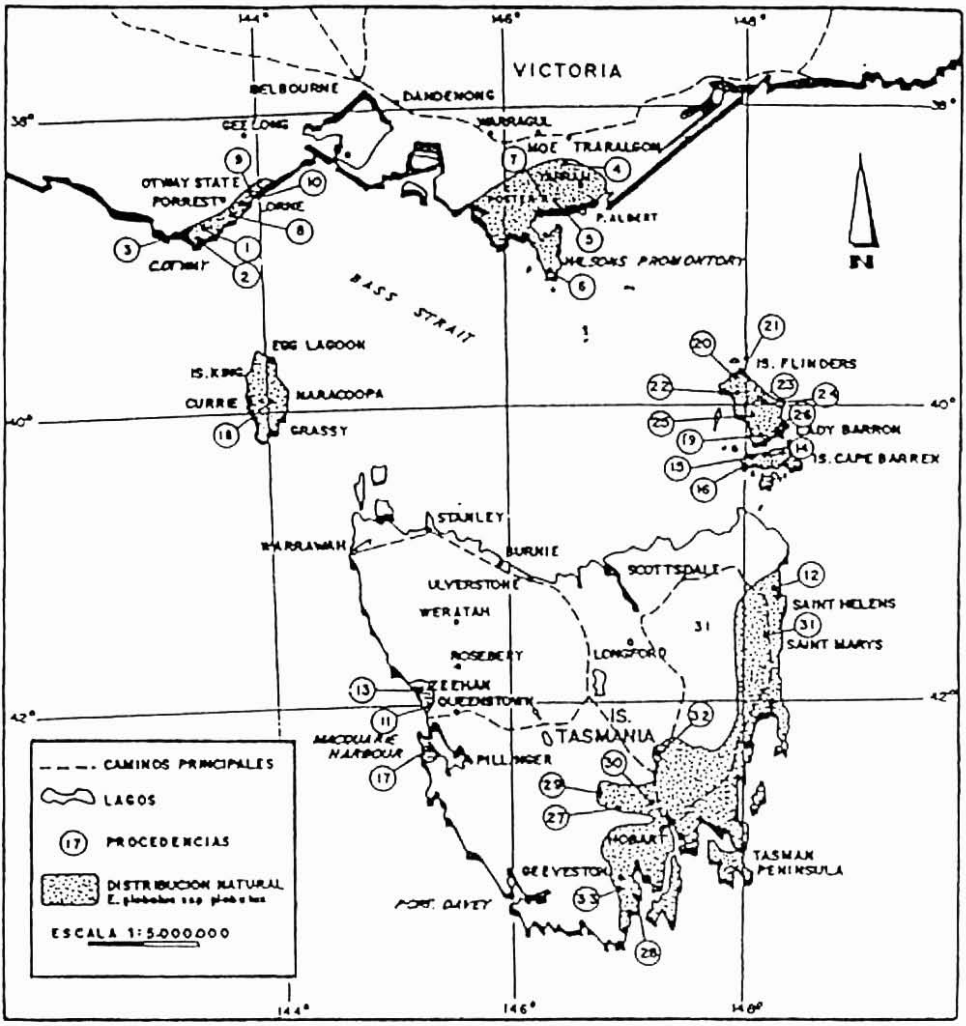

Figura Nº 1 **DISTRIBUCIÓM NATURAL DE LA ESPECIE**
Eucalyptus globulus ssp. globulus.

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Cuadro Nº 1

ANTECEDENTES SOBRE LAS PROCEDENCIAS DE *Eucalyptus globulus* ssp. *globulus* INTRODUCIDAS EN CHILE.

PROCE-DENCIA	LUGAR DE ORIGEN		FAM. Nº	LATITUD (L.S.)	LONGITUD (L.W.)	ALTITUD (msnm)
1	W Apollo Bay	VIC	4	38°46'	143°32'	200
2	SW Apollo Bay	VIC	7	30°49'	143°34'	145
3	Otway State Forest	VIC	18	38°25'	143°27'	150
4	Jeeralang North	VIC	30	38°19'	146°36'	220
5	Hodgson Hedley	VIC	4	38°38'	146°30'	20
6	Wilsons Promontory	VIC	9	39°08'	146°25'	60
7	N Toora	VIC	2	38°37'	146°21'	180
8	W Kennett River	VIC	6	38°39'	143°48'	250
9	NW of Lorne	VIC	6	38°31'	143°57'	210
10	W Lorne	VIC	9	38°31'	143°56'	210
11	Badgers CK Quarry	TAS	8	41°59'	145°18'	120
12	Binalong Bay	TAS	6	41°16'	148°18'	120
13	Litle Henty River	TAS	6	41°56'	145°12'	10
14	N Cape Barren Is.	TAS	5	40°22'	148°13'	20
15	NW Cape Barren Is.	TAS	7	40°21'	148°07'	20
16	SW Cape Barren Is.	TAS	4	40°26'	148°03'	40
17	Macquarie Harbour	TAS	3	42°20'	145°20'	20
18	King Island	TAS	4	40°00'	144°00'	60
19	S Flinders Island	TAS	3	40°14'	148°08'	120
20	NW Flinders Island	TAS	1	39°46'	147°52'	20
21	N Flinders Island	TAS	3	39°45'	147°57'	40
22	Central Flinders I	TAS	5	39°55'	147°57'	40
23	Central Flinders I	TAS	8	40°02'	148°01'	190
24	E Flinders Island	TAS	1	39°59'	148°11'	60
25	Central Flinders I	TAS	3	40°04'	148°04'	200
26	S Flinders Island	TAS	3	40°16'	148°10'	40
27	Moogara	TAS	16	42°47'	146°55'	500
28	NW Dover	TAS	5	43°16'	146°59'	190
29	Ellendale	TAS	5	42°38'	146°42'	460
30	New Norfolk	TAS	4	42°43'	147°09'	300
31	N St. Marys	TAS	5	41°34'	148°12'	400
32	SW Jericho	TAS	5	42°25'	147°16'	500
33	S Geeveston	TAS	7	43°12'	146°54'	250
34	N Geeveston	TAS	2	43°08'	146°57'	200
35	Tasmania Peninsula	TAS	4	43°04'	147°50'	20

VIC : Victoria.
TAS : Tasmania.

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Establecimiento de los ensayos.

Los ensayos fueron establecidos en 1989, en tres sitios, con condiciones climáticas y de suelo bastante diversas. La ubicación de los ensayos se presenta en el Cuadro Nº 2.

Cuadro Nº 2.

UBICACIÓN DE LOS ENSAYOS DE Eucalyptus globulus ssp. globulus ESTABLECIDOS EN CHILE.

ENSAYO	PREDIO	REGION	PROVINCIA	LATITUD	LONGITUD
1	San Agustín	VII	Cauquenes	35°54′	72°07′
2	Los Hermanos	VIII	Arauco	37°41′	73°66′
3	Los Copihues	X	Valdivia	40°01′	73°00′

El predio San Agustin, donde se encuentra instalado el ensayo 1, presenta condiciones bastante marginales para la especie, ya que se caracteriza por una precipitación reducida (717 mm/año) y lo más importante, por un largo periodo seco, que puede extenderse de 4 a 6 meses.

El segundo ensayo, denominado Los Hermanos, en las cercanias de Cañete, se encuentra en una zona de condiciones generales favorables para el crecimiento de la especie. A pesar de esto, el sitio presenta ciertas limitaciones de suelo, producidas por una larga historia de producción agrícola y pecuaria.

El tercer ensayo, ubicado en el fundo Los Copihues, cerca de Paillaco en la X región, presenta buenas condiciones de suelo, alta pluviometria, pero con la limitación de bajas temperaturas. También esta establecido en tierras que anteriormente fueron empleadas en la producción pecuaria.

Los tres ensayos fueron establecidos empleando técnicas intensivas de tratamiento de suelo, fertilización y control de competencia, con plantas producidas

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en el vivero de INFOR, en Santiago. Lamentablemente en el ensayo 3 los cuidados posteriores no fueron suficientes para controlar adecuadamente la competencia del pasto, lo cual, sumado al efecto de las heladas, produjo daños de importancia en este ensayo. Los otros dos ensayos se han mantenido en forma adecuada.

Diseño experimental y mediciones.

El diseño experimental empleado en los tres ensayos fue el de "Bloques de familias compactas" o de parcelas divididas, en donde la parcela principal es la procedencia. Las familias se distribuyen al azar en subparcelas de cuatro plantas en línea, dentro de cada procedencia. El ensayo consta de 10 repeticiones, en igual número de bloques. El ensayo fue rodeado por dos líneas de aislación.

Los ensayos han sido medidos en 4 ocasiones, dentro de los dos primeros meses después de la instalación y a los 8, 20 y 48 meses, aproximadamente. En las primeras mediciones se midió la altura total y diámetro del cuello, para luego, en las dos últimas mediciones, reemplazar esta última por el diámetro a la altura del pecho (DAP).

Análisis Estadístico

Para determinar las diferencias en el desarrollo de las plantas, tanto a nivel de procedencias como de familias, las variables analizadas: altura total (ALT), diámetro a 1.3 m (DAP) y volumen (VOL) se sometieron a análisis de varianza. En caso de existir diferencias significativas, las comparaciones entre medias se efectuaron por la prueba de Tukey. Los datos analizados corresponden a la cuarta medición del ensayo, efectuado 4 años despúes del establecimiento. El volumen fue estimado empleando la función propuesta por Opie (1976).

En el caso del ensayo Los Copihues (3) después de analizar el efecto bloque, se decidio eliminar 1 bloque de la evaluación.

También como parte del análisis se estima la interacción genético-ambiental, tanto de las procedencias como de las familias, mediante el modelo estadístico (ajustado previamente el efecto bloque dentro de cada ensayo):

$$y_{ijkl} = \mu + P_i + F(P)_{ij} + S_k + P_i \times S_k + F_j \times S_k + e_{ijkl}$$

donde:

P_i = Efecto fijo de la i-ésima Procedencia (i = 1, .., 40).

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$F(P)_{ij}$ = Efecto fijo de la j-ésima familia dentro de la i-ésima procedencia (j = 1, .., 230).
S_k = Efecto fijo del k-ésimo sitio (k = 1, ..., 3).
$P_i \times S_k$ = Interacción entre procedencia y sitio.
$F_j \times S_k$ = Interacción entre familia y sitio.
e_{ijkl} = Error experimental.

Además se estimaron la heredabilidad (h^2), las correlaciones genéticas y fenotípicas entre las variables incluidas en el análisis: ALT, DAP y VOL.

El modelo estadístico utilizado en la estimación de los parámetros genéticos, dentro de cada ensayo, fue un modelo anidado completamente al azar, considerando los siguientes factores (Becker, 1984):

$$y_{ikjl} = \mu + b_i + p_j + f(p)_{jk} + b_i \times p_j + b_i \times f(p)_{jk} + e_{ijkl}$$

donde:

b_i = Efecto aleatorio del i-ésimo bloque.
p_j = Efecto aleatorio de la j-ésima procedencia.
$f(p)_{jk}$ = Efecto aleatorio de la k-ésima familia dentro de la j-ésima procedencia.
$b_i \times p_j$ = Efecto de la interacción entre bloque y procedencia.
$b_i \times f(p)_{jk}$ = Efecto de la interacción entre bloque y familia dentro de procedencia.
e_{ijkl} = Error experimental.

La heredabilidad individual fue estimada de acuerdo a la formula propuesta por Whitemann **et al.**(1992), donde :

$$h^2 = \frac{2.86 \times \text{Varianza f}}{\text{Varianzas (b + f + b x f(p) + Error)}}$$

El factor 2.86 asume que aproximadamente el 20% de las progenies, producto de la polinización abierta en el bosque nativo de donde se colectaron las semillas, provienen de autopolinización, lo que determina un coeficiente de parentesco de 0.35.

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RESULTADOS Y DISCUSION.

Procedencias

Con el fin de tener una visión más global de los resultados de crecimiento de los distintos origenes de la semilla en Australia, se agruparon las areas de colección, conformando 16 procedencias de las 35 originales. A estas se agregan otros lotes de semillas, de procedencias locales y otras especies relacionadas. Los resultados obtenidos en los tres lugares de ensayo se presentan en los cuadros siguientes.

Los resultados que se presentan en el Cuadro Nº 3 indican un buen desarrollo y sobrevivencia de la mayoría de las procedencias ensayadas, especialmente si se considera las dificiles condiciones climáticas imperantes en el área.

Cuadro Nº 3.

SOBREVIVENCIA Y DESARROLLO DE LAS PROCEDENCIAS DE Eucalyptus globulus ssp. globulus, ENSAYADAS EN SAN AGUSTIN, VII REGIÓN.

PROCEDENCIA	SOBREV. (%)	ALTURA (m)	DIAMETRO (cm)	VOLUMEN (m^3)	TUKEY
Testigo APPM	95	7.91	7.33	0.0176	
Otway	84	7.95	6.91	0.0164	
Geevestón-Dover	85	8.13	6.90	0.0162	
Testigos Nacionales	88	7.83	6.70	0.0149	
Flinders Central	90	7.78	6.79	0.0147	
Cape Barren Island	93	7.71	6.77	0.0144	
Jericho Tasmania	93	7.25	6.77	0.0140	
Tasmania Noreste	80	7.26	6.48	0.0136	
Lorne	89	7.83	6.43	0.0133	
Tasmania Central	84	7.68	6.46	0.0133	
Testigos Subespecies	92	7.47	6.39	0.0131	
Flinders Island Sur	90	7.41	6.48	0.0130	
Jeeralang	90	7.58	6.29	0.0129	
Toora	62	7.43	6.14	0.0126	
Flinders Island Norte	87	7.07	6.35	0.0120	
Tasmania Oeste	87	6.93	5.81	0.0100	
Tasmania Peninsula	62	6.61	5.78	0.0099	
King Island	79	6.74	5.64	0.0091	
Wilsons Promontory	70	5.15	4.24	0.0040	
PROMEDIOS	84	7.56	6.45	0.0135	

Los mejores resultados corresponden a uno de los lotes testigo, procedente de un huerto semillero en Tasmania (APPM), reflejando la ganancia de una selección previa. A pesar de esto, no se encuentran diferencias significativas con las

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procedencias Otway (Victoria) y Geeveston-Dover (Tasmania), que son las que presentan los mayores desarrollos en altura. Es importante destacar que los testigos nacionales no difieren significativamente de las mejores procedencias.

Finalmente, es interesante destacar los resultados de la procedencia Jericho, ya que es un área de condiciones muy semejantes a las del área de estudio. Es probable que esta procedencia, con un crecimiento levemente inferior, sea la que, a la larga, se adapte mejor a esta zona.

En el Cuadro Nº 4, se presentan los resultados del ensayo establecido en el predio Los Hermanos, en Arauco.

Cuadro Nº 4.

SOBREVIVENCIA Y DESARROLLO DE LAS PROCEDENCIAS DE *Eucalyptus globulus* ssp. globulus, ENSAYADAS EN LOS HERMANOS, VIII REGIÓN.

PROCEDENCIA	SOBREV. (%)	ALTURA (m)	DIAMETRO (cm)	VOLUMEN (m^3)	TUKEY
Testigos Nacionales	99	8.86	8.61	0.0263	
Flinders Island Central	97	8.55	7.73	0.0198	
Geeveston-Dover	95	8.56	7.67	0.0196	
Testigos APPM	100	8.60	7.79	0.0194	
Lorne	96	8.65	7.48	0.0189	
Otway	96	8.39	7.41	0.0185	
Jeeralang	96	8.50	7.29	0.0175	
Cape Barren Island	96	8.33	7.41	0.0175	
King Island	95	7.91	7.28	0.0166	
Testigos Subespecies	89	8.34	7.06	0.0164	
Tasmania Oeste	96	8.09	7.19	0.0161	
Tasmania Peninsula	78	8.14	7.14	0.0158	
Toora	64	8.23	6.81	0.0158	
Tasmania Central	95	7.87	7.14	0.0156	
Flinders Island Sur	94	8.04	6.97	0.0153	
Flinders Island Norte	98	7.86	6.92	0.0150	
Tasmania Noreste	96	7.75	6.94	0.0148	
Jericho Tasmania	92	7.68	6.73	0.0134	
Wilsons Promontory	91	5.52	4.75	0.0048	
PROMEDIOS	95	8.24	7.27	0.0172	

Los mejores resultados en este ensayo corresponden a los testigos nacionales, que presentan diferencias estadísticamente significativas con las demás

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procedencias en ensayo. Esta semilla fue colectada en áreas productoras por Bosques Arauco, en la misma zona donde esta establecido el ensayo. Es importante señalar, sin embargo, que estas plantas fueron producidas en macetas de volumen casi 5 veces superior a las del resto de las plantas. Esta ventaja se manifestó en un mayor desarrollo desde el comienzo (Infante y Prado, 1991).

Entre las cinco mejores procedencias nuevamente se encuentran Central Flinders Is., Geeveston-Dover, Otway y el testigo de huerto semillero (APPM). En este caso se manifiesta la importancia de las analogías climáticas en la selección de especies y procedencias, al observar los resultados de la procedencia Jericho, que se contaba entre las mejores en el ensayo establecido en condiciones de semiaridez y aqui presenta los peores resultados junto a Wilsons Promontory.

Finalmente, en el Cuadro Nº 5 se presentan los resultados obtenidos en Los Copihues, provincia de Valdivia. A pesar de que las condiciones de precipitación y suelo son favorables, los crecimientos en este ensayo son significativamente inferiores a los alcanzados en los anteriores. Esto en alguna medida se debe a las dificultades encontradas para controlar la maleza competidora.

Cuadro Nº 5.

SOBREVIVENCIA Y DESARROLLO DE LAS PROCEDENCIAS DE *Eucalyptus globulus* ssp. *globulus*, ENSAYADAS EN LOS COPIHUES, VALDIVIA.

PROCEDENCIA	SOBREV. (%)	ALTURA (m)	DIAMETRO (cm)	VOLUMEN (m^3)	TUKEY
Tasmania Oeste	79	5.54	5.33	0.0079	
King Island	81	4.98	4.92	0.0071	
Cape Barren Island	84	5.25	5.00	0.0065	
Geeveston-Dover	66	5.03	4.72	0.0059	
Flinders Island Central	69	5.00	4.84	0.0059	
Testigos Nacionales	61	4.99	4.57	0.0057	
Lorne	78	5.14	4.43	0.0056	
Otway	86	5.00	4.46	0.0054	
Tasmania Noreste	78	4.61	4.42	0.0052	
Tasmania Paninsula	65	4.84	4.37	0.0051	
Jeeralang	84	5.10	4.32	0.0050	
Flinders Island Sur	71	4.87	4.49	0.0049	
Testigos APPM	78	4.95	4.74	0.0048	
Toora	43	5.09	4.14	0.0047	
Flinders Island Norte	68	4.46	4.19	0.0045	
Tasmania Central	88	4.62	4.15	0.0044	
Jericho Tasmania	74	4.15	3.74	0.0029	
Wilsons Promontory	62	3.01	2.64	0.0012	
PROMEDIOS	77	4.93	4.47	0.0054	

Es interesante destacar los cambios que se producen en relación a los ensayos anteriores. Aparecen entre las mejores procedencias las Islas King y Cape

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Barren y las aisladas poblaciones del oeste de Tasmania, Little Henty River y Maquarie Harbour. Se mantienen Geeveston-Dover y el centro de Flinders Is. Otway, en cambio, desciende a una segunda posición, al igual que la semilla de huerto, que baja considerablemente.

Estos resultados son muy coincidentes con los obtenidos por Volker y Orme (1988) en un ensayo establecido en Geeveston (43° 12' LS), Tasmania, donde entre las mejores procedencias también se cuentan Tasmania Oeste, King Island, Geeveston y Flinders Island.

Si se analiza el resultado de las procedencias en los tres ensayos, queda de manifiesto que existen tres áreas que indudablemente producen árboles de mayor desarrollo, que son Geeveston-Dover (Tasmania), Otways (Victoria) y el centro de Flinders Island, en el Estrecho de Bass. Esto coincide plenamente con los resultados de los análisis realizados en Australia (B. Potts y R. Griffin, Comunicación personal, 1993) donde también se destaca la procedencia Jeeralangs, que en estos ensayos hasta el momento no se encuentra entre las mejores.

Familias.

El resultado del análisis a nivel de familias se resume en el Cuadro Nº 6, que presenta los valores correspondientes a las 5 mejores familias en cada ensayo.

A nivel de familias es importante destacar como ciertas procedencias predominan en los distintos lugares de ensayo, Por ejemplo, en San Agustín (Cauquenes) las mejores 4 familias corresponden a Otway y la quinta a Geeveston. Al considerar las 25 mejores, 11 corresponden a Otway y sólo 3 a Geeveston. En Valdivia predominan las familias Little Henty River, del Oeste de Tasmania, con 6 representantes entre las primeras 25 familias. En cambio en Arauco, las tres mejores procedencias estan representadas. Entre las 25 mejores hay 6 de Otway, 5 del centro de Flinders Is. y 4 Geeveston, agregándose la procedencia local.

A pesar de la variabilidad que existe entre los 3 ensayos, es importante destacar la familia 215 de Geeveston, con muy buenos desarrollos en los 3 lugares y la familia 7 de Parker Rd. Otway, entre las cinco mejores familias en 2 de los ensayos.

En el caso de Arauco se puede ver como ha variado la ubicación relativa de las familias entre los 20 meses y los 4 años. En la primera evaluación (Infante y Prado, 1991) entre las 20 mejores familias predominaban las del centro de Flinders Is., Lorne (VIC) y Geeveston (TAS). A los 4 años a estas procedencias se agrega Otway (VIC), quedando cada una representada por 4 familias.

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De las 20 familias seleccionadas en 1991, sólo 14 continuan entre las mejores. La mejor familia a los 20 meses (162 de Flinders Is.) ya no se encuentra representada. A pesar de que en los 2 años transcurridos se produjeron cambios de importancia, especialmente por la entrada de un mayor número de familias de Otway, la selección temprana permitiría incluir al menos un 80% de las familias que a los 4 años se encuentran entre las 20 mejores.

Cuadro Nº 6.

CRECIMIENTO DE LAS MEJORES 5 FAMILIAS POR ENSAYO.

ENSAYO	PROCEDENCIA N°	FAMILIA N°	ALTURA (m)	DIAMETRO (cm)	VOLUMEN (m^3)
1	Otway SF - 3	27	9.02	7.87	0.023
	SW Apollo - 2	9	8.67	7.78	0.023
	Otway SF - 3	7	8.69	7.93	0.022
	SW Apollo - 2	21	8.67	7.94	0.022
	S Geeveston-33	215	9.04	7.85	0.021
2	Test. Nac. -36	227	9.77	10.42	0.038
	C Flinders -25	167	8.68	8.65	0.024
	SW Apollo - 2	7	8.84	8.26	0.024
	S Geeveston-33	215	9.12	8.38	0.024
	Jeeralang - 4	52	9.11	8.32	0.023
3	Litle Henty-13	118	6.12	6.63	0.012
	S Geeveston-33	215	6.48	6.52	0.012
	Litle Henty-13	115	6.45	6.63	0.011
	Hodgson H. - 5	60	6.67	6.08	0.011
	Litle Henty-13	116	6.27	6.16	0.010

(*): Ver Cuadro Nº 1.

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Esto concuerda con lo expresado por Bennet **et al.**, 1989 (Citado por Woolaston **et al.** 1991) quienes señalan que a los 20 meses ya se puede identificar a los "perdedores" y a un grupo de "ganadores". De acuerdo a estos resultados, para tener mayor probabilidad de incluir a las mejores familias, a esta edad sería necesario seleccionar a más de 20 familias.

Interacción Genotipo - Ambiente.

Como resultado de este análisis se demostró que ambas interacciones, procedencia x sitio y familia x sitio fueron altamente significativas para las tres variables estudiadas ($p \leq 0.001$), lo que refleja los cambios en las mejores procedencias según lugar de ensayo, como se comentaba en los puntos anteriores.

Es bastante lógico esperar que dadas las variaciones de sitio existentes entre los ensayos, sea necesario desarrollar distintas líneas de mejoramiento.

Parametros genéticos.

Los parámetros genéticos estimados en las poblaciones de **Eucalyptus globulus ssp. globulus** dentro de cada ensayo fueron: la heredabilidad individual y correlación genética. Además, se entregan las correlaciones fenotípicas entre las variables estudiadas: ALT, DAP y VOL.

En el Cuadro Nº 7 se entregan las heredabilidades individuales y sus errores estandares (Becker, 1984) de las variables ALT, DAP y VOL. La heredabilidad promedio ponderada y sus errores estandares se calcularon segun el método de Cunningham **et al.** (1977).

Las heredabilidades calculadas para las alturas son moderadas, variando de 0.17 a 0.27, con errores estandares reducidos y un valor promedio de 0.20. Volker **et al.** (1990) en Tasmania, calculan heredabilidades de 0.12 ± 0.05 a la edad de 6 años. Borralho **et al.** (1992b), en cambio, calculan heredabilidades muy superiores para esta variable, con valores de 0.29 ± 0.09 y 0.34 ± 0.20, en ensayos de 4 y 6 años de edad, respectivamente. Sin embargo, la consistencia de estos valores es discutible, debido al alto error estandard estimado. Otra diferencia con la estimación hecha en este estudio es el porcentaje de autocruzamiento asumido. Borralho **et al.** (1992a y b) asumen solo un 10% de autopolinización, contra un 20% que se asume en este estudio.

Las heredabilidades calculadas para el DAP son ligeramente superiores a las de ALT, especialmente en el ensayo establecido en Arauco, donde se produce una

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Cuadro Nº 7

HEREDABILIDAD INDIVIDUAL POR ENSAYO PARA ALTURA TOTAL, DIÁMETRO Y VOLUMEN EN ENSAYOS DE Eucalyptus globulus ssp. globulus.

ENSAYO	ALTURA $h^2 \pm$ s.e.	DIAMETRO $h^2 \pm$ s.e.	VOLUMEN $h^2 \pm$ s.e.
1	0.18 0.03	0.17 0.03	0.12 0.02
2	0.27 0.04	0.34 0.05	0.32 0.04
3	0.17 0.03	0.19 0.03	0.14 0.03
PROMEDIO	0.20 0.02	0.21 0.02	0.15 0.02

diferencia considerable. En este caso la h^2 alcanza un valor de 0.34, con un error estandard moderado. Volker **et al.** (1990) calculan un valor de 0.24 ± 0.07 para esta variable. Woolaston **et al.** (1991) calculan h^2 muy bajas, en cuatro sitios en Victoria (Australia) en ensayos de 4 años de edad (0.06 a 0.16).

Finalmente, en el VOL la variabilidad entre los tres ensayos es mayor, con un rango de 0.12 a 0.32, entre San Agustin (1) y Los Hermanos (2). La única referencia para **E. globulus** está dada por Volker **et al.** (1990), quienes calcularon un valor de 0.19 ± 0.07.

Es importante destacar que las heredabilidades obtenidas en el ensayo establecido en Arauco (2) fueron consistentemente más altas que las de los otros ensayos, debido a una significativa reducción en el componente de varianza entre bloques, revelando mucha homogeneidad en el sitio y en las condiciones en que se ha mantenido el ensayo.

En los ensayos establecidos en Cauquenes y Valdivia las heredabilidades son moderadas, especialmente para el volumen, lo que sugiere que se pueden esperar ganancias también moderadas a partir de la selección individual de árboles. En cambio, los resultados obtenidos en Arauco, con valores entre 0.27 y 0.34, indican que las variables estan bajo un considerable control genético aditivo, por lo que podrían esperarse considerables ganancias a partir de la selección individual. En los

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casos anteriores, en cambio, la mejora sería más eficiente a través de la selección a nivel de familias.

Sin duda que es necesario volver sobre estos cálculos, ya que se producen cambios en la medida que los árboles crecen, ojalá empleando métodos más robustos, como el método BLUP (Best Lineal Unbiased Predictor) (White y Hodges, 1988).

Las correlaciones genéticas (r_G) promedios ponderadas de los tres ensayos y las correlaciones fenotípicas, calculadas como simple promedio aritmético, se entregan en el cuadro Nº 8.

Cuadro Nº 8.

CORRELACIONES GENÉTICAS (SOBRE LA DIAGONAL) Y FENOTÍPICAS (BAJO LA DIAGONAL) ENTRE LAS CARACTERÍSTICAS DE CRECIMIENTO: ALTURA TOTAL, DIÁMETRO Y VOLUMEN DE Eucalyptus globulus ssp. globulus.

CARACTERISTICA	CORRELACIONES ALTURA	DIAMETRO	VOLUMEN
ALTURA		0.94 ± 0.01	0.94 ± 0.01
DIAMETRO	0.86		0.99 ± 0.01
VOLUMEN	0.81	0.91	

Las correlaciones genéticas entre las variables de crecimiento estudiadas son altas y con errores estandard muy reducidos. Las correlaciones entre altura y diámetro contrastan con los obtenidos por Volker **et al.** (1990), que son muy inferiores ($r_{G\ ALT\text{-}DAP}$= 0.55 ± 0.02; $r_{G\ ALT\text{-}VOL}$= 0.70 ± 0.15). En cambio, la correlación entre diámetro y volumen son muy semejantes.

Las correlaciones fenotípicas también son altas y positivas, pero en general menores que las correlaciones genéticas. Tendencias y magnitudes semejantes entre estas mismas variables dasométricas han reportado Volker **et al.** (1990) y Borralho **et al.** (1992a).

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Al existir estas correlaciones, el mejoramiento en base a una de estas características asegura también una ganancia consistente en las otras variables relacionadas.

CONCLUSIONES.

Aún cuando la especie **Eucalyptus globulus ssp. globulus** no presenta gran variabilidad en comparación con otras especies (por ejemplo, **E. camaldulensis; E. nitens**), de este estudio se concluye que existen tres procedencias que definitivamente producen árboles superiores en relación a las variables analizadas, particularmente en volumen. Estas son Otways (Victoria), Geeveston (Tasmania) y Central Flinders Island, en el Estrecho de Bass, que en los tres lugares de ensayo están entre las mejores procedencias.

En el ensayo establecido en Valdivia, sin embargo, se producen ciertas variaciones, que agregan las procedencias del Oeste de Tasmania (Little Henty River) y King Island, lo cual coincide con otros estudios realizados al sur de Tasmania, en latitudes semejantes (Volker **et al.**, 1990).

A nivel de familias los mejores resultados por lo general corresponden a las mismas procedencias. En el ensayo situado más al norte, Cauquenes, predominan las familias de Otways (Victoria) en cambio en Valdivia predominan las del Oeste de Tasmania. En Arauco, que se encuentra en una situación intermedia, no existe un procedencia bien definida de la cual provengan las familias dominantes. Las 5 mejores familias proceden de 5 procedencias distintas. Sólo una familia, procedente de Geeveston (Tasmania) está presente entre las cinco mejores familias de los tres ensayos. Este resultado, en conjunto por el logrado por la procedencia en general, señala a esta fuente de semilla como la de mejor adaptación a las diversas condiciones que representan los tres lugares de ensayo.

Las heredabilidades estimadas son de medias a altas, en comparación con otros estudios, lo cual sugiere que se pueden obtener considerables ganancias mediante la selección de árboles individuales, aún cuando, en algunos casos la selección a nivel de familias aún puede generar ganancias considerables.

En este caso se han analizado las variables de crecimiento, lo cual no necesariamente implica que en el programa del Instituto se vayan a establecer índices de selección basados sólo en estas características. Sin duda que otras variables, dependiendo de las direcciones que vaya tomando el programa, deberán ser consideradas. Variables tales como densidad, rendimiento pulpable, resistencia al frío, resistencia a plagas y enfermedades podrán ser evaluadas próximamente.

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DESARROLLO Y PARAMETROS GENETICOS DE *Eucalyptus nitens* EN EL CENTRO Y SUR DE CHILE.

Carlos Alvear S. (*)
José A. Prado D. (**)

RESUMEN.

*Se analiza el comportamiento de 187 familias de **Eucalyptus nitens** que cubren casi todo el rango de dispersión natural de la especie en diferentes lugares del país, desde la VIII a la XI región.*

Las características de crecimiento evaluadas a los 24 meses de edad fueron: la altura total, diámetro del cuello y la estimación de biomasa mediante el D^2H.

*Las mejores procedencias de semilla son de Victoria Central (Toorongo, Rubicon y Macalister), mientrás que la procedencia con menor crecimiento fue Errinundra, hoy día denominada **Eucalyptus denticulata**.*

A nivel de familias predominaron también las provenientes de Victoria Central.

Se estimaron heredabilidades individuales para las características de crecimiento, resultando en general de valores bajos, sugiriendo por lo tanto, escazas ganancias que pueden lograrse en base a selección individual, señalándose la selección a nivel de familias o índice de selección como la estrategia más adecuada.

También se estimaron las correlaciones genéticas y fenotípicas entre estas variables de crecimiento.

La base de un proyecto de mejoramiento genético de mediano plazo, deberá estar constituido, principalmente por familias de Victoria Central.

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INTRODUCCION.

La especie **Eucalyptus nitens** fue introducida a Chile por el Instituto Forestal hace casi 30 años. Sin embargo, sólo ha adquirido importancia económica durante esta década, donde ha surgido como la especie más adecuada para la plantación en áreas donde la especie tradicionalmente plantada, **Eucalyptus globulus ssp. globulus**, presenta serias limitaciones por causa del frío.

Eucalyptus nitens es una especie originaria de los Estados de Victoria y New South Wales, en Australia, donde, a pesar de ocupar áreas bastante reducidas, presenta una gran dispersión.

E. nitens ha demostrado ser adaptable a diversas condiciones de suelos y climas y de gran velocidad de crecimiento, por lo que se ha convertido en una especie de interés económico no sólo en Chile, si no que también en Australia; Sud Africa y otros países.

Por lo disperso de su distribución natural, la especie presenta considerables variaciones (Cook and Ladiges, 1991; Pederick, 1979) las que se reflejan no sólo en su morfología externa, si no que también en características tales como tasa de crecimiento, densidad de la madera y resistencia al frío.

Las procedencias de Victoria Central presentan hojas juveniles más anchas, glaucas, que persisten por largo tiempo, lo cual confiere a estas plantas una mayor velocidad de crecimiento inicial y supuestamente un mayor grado de tolerancia al frió (King y Wilcox, 1988; Tibbits y Reid, 1987).

La procedencia que más difiere es la de Errinundra, al este de Victoria. Tal es su variación morfológica que recientemente ha sido reclasificada como **Eucalyptus denticulata** (Cook y Ladiges, 1991) debido al borde denticulado de sus hojas. Representantes de esta nueva especie también se encuentran presentes en algunas poblaciones en Toorongo Plateau, Victoria Central (Pederick, 1979). En este trabajo se le dará el tratamiento de procedencia.

Por la importancia económica que la especie ha ido adquiriendo en el país, el Instituto Forestal decidió establecer algunos ensayos de procedencias y progenies, en los cuales están representadas casi todas las poblaciones naturales, ya que sólo falta la procedencia denominada Macalister (Victoria).

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El presente trabajo entrega los primeros resultados obtenidos de estos ensayos, a casi dos años de su establecimiento. Se analiza el desarrollo logrado en 5 localidades y se calculan algunos parámetros genéticos y fenotípicos.

MATERIAL Y METODO.

Antecedentes de la Colección de Semillas.

Los ensayos incluyen 187 familias, de 16 lugares de colección, representando 5 de las 6 procedencias tradicionalmente definidas para esta especie, constituyendo una muestra importante de sus áreas de distribución natural, las que se muestran en la Figura Nº1. El detalle de las procedencias se entrega en el Cuadro Nº1. La colección fue realizada por el Centro de Semillas de CSIRO, Australia, en los años 1987 y 1988.

Cuadro Nº1

ANTECEDENTES SOBRE EL ORIGEN DE LAS PROCEDENCIAS Y FAMILIAS DE *Eucalyptus nitens* ENSAYADAS POR EL INSTITUTO FORESTAL.

PROCE-DENCIA	LUGAR DE ORIGEN		FAMILIA Nº	LATITUD (L.S.)	LONGITUD (L.W.)	ALTITUD (msnm)
1	Majors Point Ebor	NSW	3	30°25'	152°25'	1450
2	Barrington Tops	NSW	5	31°55'	151°30'	1450
3	Tallaganda S.F	NSW	2	35°48'	149°31'	1280
4	Tallaganda S.F	NSW	5	35°48'	149°31'	1250
5	Tallaganda S.F	NSW	10	35°52'	149°30'	1105
6	Tallaganda S.F	NSW	6	35°52'	149°28'	1417
7	Anembo Tallaganda	NSW	1	35°54'	149°30'	1300
8	Tallaganda	NSW	9	36°00'	149°54'	1050
9	Brown Mt.Nimmitabel	NSW	6	36°32'	149°28'	1000
10	Glenbog State F	NSW	4	36°38'	149°24'	1050
11	Errinundra S.F	VIC	4	36°13'	148°54'	1000
12	Errinundra S.F	VIC	8	37°15'	149°02'	950
13	Errinundra Plateau	VIC	2	37°21'	148°51'	1000
14	Toorongo Plateau	VIC	24	37°47'	146°16'	900
15	Toorongo Plateau	VIC	6	37°54'	146°00'	1000
16	Rubicon	VIC	11	37°18'	145°49'	1000

NSW : New South Wales
VIC : Victoria
msnm: Metros sobre el nivel del mar

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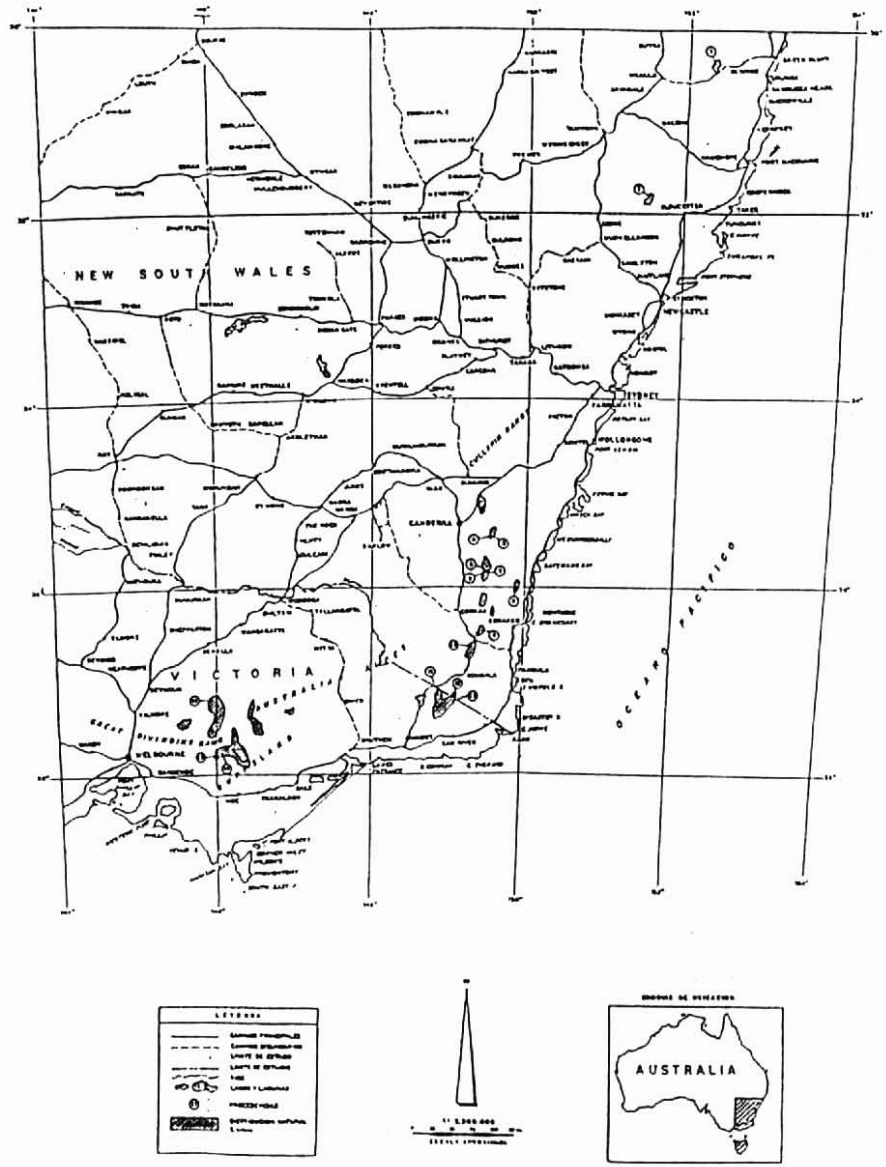

Figura Nº 1: **DISTRIBUCIÓN NATURAL DE LA ESPECIE Eucalyptus nitens.**

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Establecimiento de los Ensayos.

Los ensayos fueron establecidos en 1990 y 1991, en las cinco localidades que se señalan en el siguiente Cuadro Nº 2.

CUADRO Nº 2

UBICACIÓN DE LOS ENSAYOS DE *Eucalyptus nitens* ESTABLECIDOS EN CHILE.

ENSAYO	PREDIO	REGION	PROVINCIA	LATITUD	LONGITUD
1	El Durazno	VIII	Ñuble	36°26'	71°35'
2	San Lorenzo	VIII	Bio-Bio	37°30'	71°45'
3	El Morro	VIII	Bio-Bio	37°55'	72°00'
4	Vista Alegre	X	Valdivia	39°60'	72°50'
5	El Bajo	XI	Aysén	45°00'	72°20'

Los ensayos fueron establecidos entre Junio y Diciembre, retardando la época de plantación en la medida que aumenta la latitud. Las plantas fueron producidas en macetas, en el vivero del Instituto Forestal.

Los ensayos se establecieron empleando prácticas silviculturales intensivas, incluyendo un control inicial de malezas y fertilización. Lamentablemente se presentaron diversos problemas en su cuidado posterior, lo que ha llevado a considerar, para este análisis, sólo parte de ellos.

Diseño Experimental.

El diseño experimental empleado en todos estos ensayos es el de "Bloques de Familias Compactas", o de parcelas divididas, en donde la parcela principal es la procedencia. Las familias se distribuyen al azar en subparcelas de 4 plantas en línea, dentro de cada procedencia. El diseño consta de 10 repeticiones en los ensayos 1,2 y 3 y de 8 en los dos restantes. Los ensayos fueron rodeados por dos líneas de aislación.

En cada uno de estos ensayos se han realizado tres mediciones, una inmediatamente después de la instalación; a los 8 meses y finalmente a los 23 meses desde el establecimiento, con ligeras variaciones entre ensayos. En cada oportunidad se midió la altura total (ALT) y el diámetro del cuello (DIA) de las plantas (5 cm sobre el nivel del suelo). Además, se emplea la variable D^2H, ya que constituye una buena estimación de la biomasa total.

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Análisis Estadístico.

Para determinar las diferencias en el desarrollo de las plantas tanto a nivel de procedencias como de familias, se realizaron análisis de varianza. Las comparaciones entre medias se efectuaron empleando la Prueba de Tukey. Los datos analizados corresponden al tercer control de los ensayos, efectuado alrededor de los 23 meses después del establecimiento. Como se mencionó anteriormente, en algunos de los ensayos sólo se consideró parte de la información, por estimarse que la inclusión de algunos bloques distorsionaría los resultados.

También, como parte del análisis se estima la interacción genético - ambiental (familia - ensayo) empleando el siguiente modelo estadístico (ajustado previamente el efecto bloque dentro de cada ensayo)

$$y_{ijkl} = \mu + P_i + F(P)_{ij} + S_k + P_i \times S_k + F_j \times S_k + e_{ijkl}$$

donde:

P_i = Efecto fijo de la i-ésima Procedencia (i = 1,..., 17).
$F(P)_{ij}$ = Efecto fijo de la j-ésima familia (j = 1,..., 190) dentro de la i-ésima procedencia.
S_k = Efecto fijo del k-ésimo sitio (k = 1,..., 5).
$P_i \times S_k$ = Interacción entre procedencia y sitio.
$F_j \times S_k$ = Interacción entre familia y sitio.
e_{ijkl} = Error experimental.

Además se estimaron la heredabilidad (h^2) y las correlaciones genéticas y fenotípicas entre las variables estudiadas: ALT, DIA Y D²H.

El modelo estadístico utilizado en la estimación de los parámetros genéticos en cada uno de los ensayos fue un modelo anidado completamente al azar, considerando los siguientes factores (Becker, 1984):

$$y_{ikjl} = \mu + b_i + p_j + f(p)_{jk} + b_i \times p_j + b_i \times f(p)_{jk} + e_{ijkl}$$

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donde:

b_i = Efecto aleatorio del i-ésimo bloque.
p_j = Efecto aleatorio de la j-ésima procedencia.
$f(p)_{jk}$ = Efecto aleatorio de la k-ésima familia dentro de la j-ésima procedencia.
$b_i \times p_j$ = Efecto de la interacción entre bloque y procedencia.
$b_i \times f(p)_{jk}$ = Efecto de la interacción entre bloque y familia dentro de procedencia.
e_{ijkl} = Error experimental.

La heredabilidad individual fue estimada siguiendo el mismo procedimiento de Whitemann **et al**. (1992), donde:

$$h^2 = \frac{2.86 \times \text{Varianza f}}{\text{Varianzas (b + f + b x f(p) + Error)}}$$

El factor 2.86 se basa en el supuesto que la tasa de cruzamiento exogámico es de aproximadamente un 80%, lo cual se considera normal en el caso de familias de polinización abierta, especialmente cuando la semilla se ha colectado en bosques nativos, lo que determina un coeficiente de parentesco de 0.35.

La h^2 y correlación genética (con sus respectivos errores estandar) promedio ponderado de todos los ensayos se calculó según Cunningham **et al**. (1977), en cambio la correlación fenotípica promedio se obtuvo del promedio aritmético simple.

RESULTADOS Y DISCUSIÓN.

Procedencias.

En el Cuadro Nº 3 se entregan los resultados de sobrevivencia (%), altura media y D^2H, para cada una de las procedencias ensayadas en cada lugar. La sobrevivencia consideró soló a aquellos bloques que fueron utilizados en el análisis.

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Cuadro Nº 3.

SOBREVIVENCIA (%), ALTURA (m) y D^2H (cm^3) POR ENSAYO DE LAS PROCEDENCIAS DE Eucalyptus nitens, AGRUPADAS POR REGION GEOGRÁFICA DE ORÍGEN EN AUSTRALIA.

REGION GEOGRAFICA	ENSAYOS														
	1			2			3			4			5		
	S%	ALT	D^2H	S%	ALT	D^2H	S%	ALT	D^2H	S%	ALT	D^2H	S%	ALT	D^2H
MAJORS POINT y BARRINGTON TOPS	70	1.8	1.825	78	1.4	1.306	84	1.4	1.443	91	1.3	423	86	1.2	682
TALLAGANDA	69	1.8	1.948	80	1.4	1.222	79	1.5	1.623	89	1.3	403	90	1.2	771
BROWN Mt. NIM-MITABEL	67	1.8	1.844	76	1.5	1.144	75	1.5	1.393	90	1.4	565	71	1.2	714
GLENBOG S.F	79	1.7	1.782	71	1.4	1.241	67	1.5	1.521	90	1.2	292	71	1.3	819
ERRINUNDRA	65	2.1	1.004	70	1.6	1.265	80	1.6	1.381	83	1.3	301	86	1.4	871
TOORONGO	71	2.0	2.435	78	1.6	1.847	83	1.8	2.372	90	1.4	467	94	1.5	1.299
RUBICON	76	2.0	2.587	81	1.6	1.932	92	1.9	2.980	86	1.5	657	92	1.3	929
TESTIGO	..	...	.....	..	...	.....	88	1.7	2.683	86	1.5	676	..	...	.....
PROMEDIOS	68	1.9	2.150	78	1.5	1.416	82	1.6	1.960	89	1.3	436	88	1.3	929

S% = Porcentaje de sobrevivencia.
ALT = Altura.
D^2H = Estimación de volumen.

El desarrollo alcanzado por la especie en las distintos localidades en general es bastante limitado, a pesar de las técnicas de establecimiento empleadas. Esto se debe a la falta de un cuidado intensivo posterior, especialmente en lo que se refiere al control de malezas. En un caso (Ensayo 1) las plantas se vieron afectadas por la

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aplicación de herbicidas. A pesar de estas limitaciones, se considera que la información lograda de estos ensayos es de considerable valor, especialmente si se eliminan los bloques que presentan problemas.

Las cifras que se entregan en el cuadro anterior revelan importantes diferencias en el desarrollo de la especie en las distintas localidades donde fue ensayada. Las razones para estas diferencias están dadas tanto por variaciones en las condiciones de clima y suelo, como por variaciones en el cuidado de los ensayos. En el caso del ensayo Vista Alegre (4) el menor crecimiento sin duda se debe a la falta de cuidados después del establecimiento; no así en el caso del ensayo El Bajo, en Aysén, donde el menor desarrollo responde a las condiciones climáticas imperantes en el área.

Al comparar el desarrollo de los árboles en cada uno de los 5 lugares de ensayo, se encuentran diferencias estadísticamente significativas entre las procedencias ($p \leq 0.001$), cualquiera sea la variable considerada (ALT, DIA o D^2H). En todos los casos las mejores procedencias corresponden a Toorongo y Rubicon, Victoria Central. En el caso del ensayo establecido en Aysén no sólo se producen diferencias significativas en el desarrollo, si no que también en la sobrevivencia. Esto deja de manifiesto la mayor resistencia al frío de las procedencias de Victoria, especialmente Toorongo, ya que esta es la principal causa de mortalidad en este ensayo.

En cuanto a las procedencias menos adaptadas, hay ciertas variaciones según los sitios. La procedencia Errinundra es, por lo general, la de menor crecimiento. Sólo en el ensayo 1, situado más al norte, supera a las procedencias de NSW.

En el Cuadro Nº 4 se entrega un promedio general del crecimiento de las distintas áreas de colección ensayadas en las 5 localidades.

Estos resultados son coincidentes con los de otros estudios realizados en los mismos rangos de latitud (Woolaston, 1991; King y Wilcox, 1988; Franklin, 1980; Pederick, **et al.**, 1979). Las procedencias de Victoria Central: Rubicon, Toorongo y Macalister, esta última no incluida en este ensayo, son las que presentan los mejores desarrollos. Estos resultados sólo difieren de los presentados por Purnell y Lundquist (1986) en Sud Africa, quienes encuentran una clara superioridad de las procedencias de N.S.W. sobre las de Victoria Central. Esto puede explicarse por la latitud (25° 30' L.S.) en que se han establecido sus ensayos.

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Cuadro Nº 4.

ALTURA, DIÁMETRO Y D^2H PROMEDIO A LA EDAD DE 23 MESES DE LOS ENSAYOS DE PROCEDENCIAS DE *Eucalyptus nitens* EN CHILE.

Nº	PROCEDENCIA	ORIGEN	ALTURA (m)	DIÁMETRO (cm)	VOLUMEN (cm^3)	TUKEY (1)
16	Rubicon	VIC	1.66	2.89	2022.7	
14	Toorongo Plateau	VIC	1.67	2.81	1522.4	
15	Toorongo Plateau	VIC	1.68	2.75	1493.9	
17	Testigo	VIC	1.57	2.83	1366.4	
11	Gap Road	VIC	1.65	2.53	1227.2	
2	Barringtons Tops	NSW	1.45	2.64	1178.1	
3	Tallaganda S.F.	NSW	1.46	2.56	1174.5	
4	Tallaganda S.F.	NSW	1.45	2.59	1097.7	
6	Tallaganda S.F.	NSW	1.44	2.49	1043.1	
5	Tallaganda S.F.	NSW	1.47	2.56	1021.2	
8	Tallaganda S.F.	NSW	1.41	2.53	1012.8	
9	Nimmitabel	NSW	1.50	2.53	989.3	
7	Tallaganda S.F.	NSW	1.48	2.57	987.4	
10	Glenbog S.F.	NSW	1.43	2.46	951.0	
13	Errinundra Plateau	VIC	1.58	2.27	887.9	
12	Errinundra	VIC	1.53	2.28	846.3	
1	Majors Point	NSW	1.39	2.38	747.4	

VIC = Victoria.
NSW = New South Wales.
(1) Barras indican grupos de medias sin diferencias significativas Prueba de Tukey ($p < 0.05$).

Es importante destacar la variabilidad que existe dentro de la procedencia Toorongo. Como lo señala Pederick (1979) y posteriormente Cook y Ladiges (1991) aquí existe un alto grado de variabilidad morfológica, debido a la presencia, en distintos grados, de la forma denticulada, propia de Errinundra. Existen áreas en que ambas formas se encuentran presentes. En otras, en cambio, los árboles se acercan a una u otra forma, pero presentando características de ambas.

La presencia de genes de la procedencia Errinundra, hoy clasificada como **Eucalyptus denticulata,** lógicamente que afecta la tasa de crecimiento y resistencia al frío de estos árboles. Así lo señalan los resultados obtenidos por Woolaston **et al.** (1991), King y Wilcox (1988) y Pederick (1993), quienes identifican áreas que dan origen a árboles con tasas de crecimiento inferiores. Entre estas se señalan

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Christmas Creek, Tanjil Bren, Mt. Toorongo, Mt. Baw Baw. Por el contrario, las áreas cercanas a Mt. Erica; Mt. St. Gwinear, Mt. Horsefall, presentan poblaciones puras de la forma normal. Esto indica que se debe ser cuidadoso en la selección de semillas de la procedencia Toorongo.

Las otras dos procedencias de Victoria Central: Rubicon y Macalister, no presentan este problema.

Familias.

La evaluación de la variabilidad entre las familias se realizó mediante un Análisis de Varianza, el cual revela que existen diferencias altamente significativas, tanto entre procedencias como dentro de una misma procedencia ($p \leq 0.001$) para las tres variables analizadas.

En el Cuadro Nº 5 se presentan los resultados de las 5 mejores familias en cada uno de los ensayos.

Estos datos revelan, a nivel de familias, que los mejores resultados corresponden a Rubicon y Toorongo Plateau. Llama la atención que en el ensayo 4 situado en la X región, también se destacan familias provenientes NSW. En los sitios mas fríos, San Lorenzo (2) y el Bajo (5) las mejores familias corresponden a Toorongo Plateau, lo cual sugiere una mayor resistencia al frío de esta procedencia.

Interacción Genotipo-Ambiente.

El análisis realizado para estimar la interacción genotipo- ambiente determinó que las interacciones fueron estadísticamente significativas para las tres variables analizadas ($p \leq 0.001$) tanto a nivel de procedencia como de familias. Esto significa que no es posible hacer programas o recomendaciones de tipo general, ya que el factor ambiental afecta de distinta forma el comportamiento de las distintas procedencias y familias. En todo caso, se trata de variaciones entre las procedencias de Victoria Central.

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Cuadro Nº 5.

Crecimiento de las mejores 5 familias por ensayo.

ENSAYO	PROCEDENCIA	Nº	FAMILIA	ALTURA (m)	DIAMETRO (cm)	D^2H (cm^3)
	Rubicon	-16	178	2,04	3,79	3.146
	Rubicon	-16	181	2,09	3,41	3.134
1	Toorongo	-15	159	2,08	3,60	2.999
	Toorongo	-14	116	2,41	3,29	2.989
	Toorongo	-14	121	2,18	3,63	2.987
	Toorongo	-14	131	1,97	3,86	3.242
	Toorongo	-15	165	1,94	3,60	2.829
2	Toorongo	-14	138	1,72	3,71	2.586
	Toorongo	-14	127	1,91	3,49	2.424
	Toorongo	-14	140	1,73	3,43	2.263
	Rubicon	-16	169	2,13	4,07	4.141
	Rubicon	-16	187	2,12	4,05	4.097
3	Toorongo	-14	129	2,01	3,90	3.727
	Rubicon	-16	173	2,05	3,93	3.693
	Toorongo	-14	138	1,95	4,05	3.589
	Rubicon	-16	170	1,59	1,87	978
	Tallaganda	- 4	25	1,35	1,80	919
4	Rubicon	-16	169	1,67	1,85	862
	Barringst.	- 2	13	1,43	1,80	850
	Brown Nimm.	- 76	76	1,51	1,85	790
	Toorongo	-15	158	1,63	3,01	1.606
	Toorongo	-14	129	1,66	2,97	1.595
5	Toorongo	-14	131	1,58	2,96	1.537
	Toorongo	-14	130	1,55	2,99	1.535
	Toorongo	-14	128	1,61	2,94	1.529

(*) Ver Cuadro Nº 1.

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Parámetros Genéticos.

Los parámetros genéticos estimados en las poblaciones de **E. nitens** dentro de cada ensayo fueron: la heredabilidad individual y correlación genética. Además se entregan las correlaciones fenotípicas entre las variables estudiadas.

En el Cuadro Nº 6 se entregan las heredabilidades (h^2) individuales y sus errores estandar para las variables ALT, DIA y D^2H, por ensayo.

Cuadro Nº 6.

HEREDABILIDAD INDIVIDUAL POR ENSAYO PARA ALTURA TOTAL, DIÁMETRO Y D^2H EN 5 ENSAYOS DE Eucalyptus nitens.

ENSAYO	ALTURA $h^2 \pm$ s.e.	DIAMETRO $h^2 \pm$ s.e.	D^2H $h^2 \pm$ s.e.
1	0.25 0.06	0.08 0.04	0.03 0.03
2	0.13 0.03	0.07 0.05	0.07 0.03
3	0.18 0.03	0.18 0.03	0.18 0.03
4	0.07 0.02	0.03 0.01	0.01 0.01
5	0.13 0.02	0.10 0.03	0.08 0.03
PROMEDIO	0.11 0.02	0.06 0.02	0.04 0.02

Las heredabilidades calculadas en general son bajas. En el caso de la altura, con la excepción del ensayo 4, se acercan a los valores dados por Woolaston **et al.** (1991) y por Whiteman **et al.** (1992). En el caso del diámetro, en cambio, sólo en los ensayos establecidos en El Morro (3) y en el Bajo, Aysén (5) los valores se acercan a los dados por Whiteman **et al.** (1992), pero están muy por debajo de los valores dados en el otro estudio, que calcula valores desde 0.18 a 0.39, en diferentes sitios ensayados. Sin duda que los bajos valores calculados en este estudio responden a la variabilidad encontrada en algunos de los ensayos.

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Por lo tanto, será necesario volver sobre estos cálculos a una edad superior. Por el momento las heredabilidades calculadas para la altura y, en un par de sitios, para el diámetro, sugieren que ganancias razonables pueden esperarse de la selección individual. La componente ambiental, hasta esta edad, resulta muy fuerte y determinante en el crecimiento de los árboles especialmente cuando estan sometidos a condiciones de manejo deficientes, como lo ocurrido en el ensayo de Vista Alegre (4), cuyas estimaciones de h^2 fueron muy inferiores al resto de los ensayos debido a un aumento relativo de las varianzas bloque y interacción bloque x familia, las que determinan un aumento de la varianza fenotípica.

La correlación genética y fenotípica promedio de los 5 ensayos se entregan en el Cuadro Nº 7. En general las correlaciones genéticas fueron mayores a las fenotípicas, salvo el caso entre altura y diámetro.

Las correlaciones genéticas y fenotípicas son bastantes altas entre las variables análizadas, lo que implica un alto componente genético responsable de la asociación entre estas características de crecimiento. Whiteman **et al**. (1992) entrega una correlación genética entre ALT y DIA de 0.92 ± 0.04, también mayor a la correlación fenotípica (0.83) en **Eucalyptus nitens** de 4 años de edad.

Cuadro Nº 7

CORRELACIONES GENÉTICAS (SOBRE LA DIAGONAL) Y FENOTÍPICAS (BAJO LA DIAGONAL) ENTRE LAS VARIABLES: ALTURA TOTAL, DIÁMETRO Y D^2H EN Eucalyptus nitens.

CARACTERISTICA	CORRELACIONES ALTURA	DIAMETRO	D^2H
ALTURA		0.65 ± 0.05	0.99 ± 0.01
DIAMETRO	0.74		0.95 ± 0.01
D^2H	0.83	0.90	

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CONCLUSIONES.

A pesar de las limitaciones que presentan algunos de los ensayos analizados, los resultados obtenidos son muy consistentes con los otros ensayos establecidos en condiciones climáticas y latitudinales semejantes.

Las procedencias que mejor se adaptan a las condiciones representadas por los ensayos son las de Victoria Central: Rubicon y Toorongo Plateau. Existen suficientes antecedentes como para agregar la procedencia Macalister entre las de buena adaptación, aun cuando no fue ensayada en esta oportunidad (Prado, 1988).

Las procedencias de New South Wales presentan desarrollos inferiores a las de Victoria Central, pero, en general, superan a la procedencia Errinundra, hoy día clasificada como **Eucalyptus denticulata.**

Los parámetros genéticos calculados, indican que podría esperarse una moderada ganancia a partir de la selección de árboles individuales. Sin duda que será necesario recalcular estos parámetros a mayor edad.

La base de un proyecto de mejoramiento genético de mediano plazo deberá estar constituida, principalmente, por familias procedentes de Victoria Central. Sin embargo, no debe descartarse el aporte de características positivas, ya sea en la forma, calidad de la madera o resistencia a plagas y enfermedades de las otras procedencias.

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