



**UNIVERSITY OF TASMANIA
FACULTY OF SCIENCE & ENGINEERING
SCHOOL OF PLANT SCIENCE**

**PROPOSAL FOR A HIGHER DEGREE BY RESEARCH PROJECT
(2001-2003)**

**THE DEVELOPMENT OF EFFICIENT SYSTEMS FOR THE
PRODUCTION AND EXPLOITATION OF INTERSPECIFIC
HYBRIDS OF *Eucalyptus globulus***

**PATRICIO ROJAS VERGARA
FOREST ENGINEER, MSc**

**SUPERVISOR
DR. BRAD POTTS
UNIVERSITY OF TASMANIA-CRCSPF
HOBART- AUSTRALIA**



**COOPERATIVE RESEARCH CENTRE
FOR SUSTAINABLE PRODUCTION FORESTRY**

**CONCEPCIÓN- CHILE
AUGUST-2.000**

1. OBJECTIVES

Aims: The project aims to develop efficient methods for the production and exploitation of *Eucalyptus globulus* hybrids. In brief the project will determine:

- (i) The optimal treatments for production of hybrid seed using *E. globulus* as the female parent. This will include using morphological and molecular markers to verify hybridity, and monitoring the level of contamination in the various treatments.
- (ii) Whether there are any reciprocal effects on hybrid performance.
- (iii) Whether changes in style length or maturation impact on the genetic composition of resulting progenies and can be used as a means of manipulating the composition of backcross generations.
- (iv) Whether there is genetic variation within *E. globulus* for the success of hybridisation and whether the success and performance under intra-specific crossing can be used as a predictor of intra-specific performance.
- (v) The genetic characteristics of F₁ and advanced generation hybrid populations.

2. BACKGROUND

There are 1.8 million hectares of *Eucalyptus globulus* ssp. *globulus* planted in the world mainly for pulp and paper production, with a growth rate of between 9 to 35 m³/year (Tibbits, 1997). Chile has the second largest *E. globulus* plantation estate in the world after the Iberian Peninsula. It has around 250,000 hectares (INFOR, 1999) with an annual growth rate of between 25 to 35 m³/year; and a basic density of the wood of 492 kg/ m³ between 8 to 10 years (Peredo, 1999).

The main factor restricting the cultivation of *E. globulus* in Chile is frost. On colder sites, *E. globulus* is replaced by *E. nitens*. However, in spite of having better growth, *E. nitens* produces less pulp per hectare due to the lower basic density of the wood and reduced pulp yield compared with *E. globulus*. There is also a general reluctance by pulp mills to use *E. nitens* pulp. There is therefore increasing interest in improving the cold tolerance of *E. globulus* to extend the area available to this high quality pulpwood species. Hybridisation with cold tolerant species is seen as one means of achieving this objective through the development of frost resistant genotypes with *E. globulus*-like growth and pulpwood characteristics.

2.1 The problem with producing hybrids of *E. globulus*.

To effectively breed and deploy hybrids it is necessary to: (i) have cost effective pollination techniques to allow large numbers of hybrid combinations to be generated and screened and (ii) have an efficient means of clonal deployment or of mass producing selected hybrid seed. In the case of *E. globulus* and many of its hybrids (e.g. *E. nitens* x *globulus* Rasmussen *et al.* 1995), cloning by either hardwood cuttings or by *in vitro* techniques is difficult, which means that intensive selection for rooting ability must be undertaken to find genotypes which clonally propagate at levels which are cost-effective for large scale deployment. The mass production of hybrid seed of outstanding full-sib families is an alternative. However until recently, this option has never been a serious possibility due to the high cost of hybrid seed production. This is partly due to the fact that most species (e.g. *E. nitens* and *E. gunnii*) which are being crossed with *E. globulus*, have markedly smaller flowers and must be used as the female parent in the cross. This is because their short pollen tubes are unable to grow the full length of the *E. globulus* style, resulting in a unilateral barrier to hybridisation with many species. This is unlikely to be due to physiological incongruity (e.g. Ellis *et al.* 1991) as seed set is frequently obtained in taxonomically wider crosses or when *E. globulus* is used as the pollen parent. The barrier is believed to be mainly structural and simply due to the pollen-tubes of the smaller flowered species being unable to grow the full length of the style of the large-flowered *E. globulus* (Gore *et al.* 1990). These smaller flowered species have less seed per flower than *E. globulus* and their more delicate flowers means that crossing is more time-consuming and thus expensive than when *E. globulus* is used as the female. Further, although F₁ hybrid seed set is often possible when the small flowered species are used as the female parent, seed set is still reduced compared to intra-specific outcrosses, possibly due to pollen tubes over shooting the ovules.

2.2 Recent developments in controlled pollination of eucalypts

Recent developments in controlled pollination of eucalypts have now opened the possibility of mass production of controlled pollinated seed of *E. globulus* and potentially a means of mass-producing hybrids using the large flowered species *E. globulus* as the female parent. Due to its large flower size *E. globulus* is probably the only eucalypt in which controlled pollinated families could be cost-effectively produced for deployment (Shelbourne 1999).

The technique traditionally used in the pollination controlled of *Eucalyptus* requires at least three visits:

- (1) emasculation at operculum shed in order to prevent selfing and isolation of the flower in order to prevent contamination from non-target pollen;
- (2) pollination with the target pollen at maximum receptivity of the stigma, 3 to 28 days after operculum shed, depending on the species and environment; and
- (3) removal of the isolation bag after the fertilisation, about 2 to 4 weeks after pollination.

In the case of *E. globulus* and many other species of eucalypts, new developments have now reduced the pollination procedure to a single visit (Cauvin 1988; Harbard *et al.* 1999, 2000; Williams *et al.* 1999). It has been found that pollination does not depend on the stigma and pollination can be just as successful when pollen is applied to the cut surface of the style at the time of emasculation or even earlier (prior to operculum shed). The style can be either transversely cut (Williams *et al.* 1999) or sliced (Harbard *et al.* 1999). Pollination appears possible when between 10 to 66% of the style is removed in *E. globulus*. Plastic or silicon tubes have also been used which slip over the style itself allowing contamination to be prevented without having to isolate the whole flower (e.g. Barbour, 1997). It is therefore unnecessary to return to the flower to remove the isolation bag.

These developments have dramatically cut the cost of controlled crossing in eucalypts and in the last few years operational crossing of *Eucalyptus globulus* in seed orchards has been successfully implemented in Chile (Harbard *et al.* 1999, 2000). The production of full-sib *E. globulus* families for deployment allows the capturing of both additive and non-additive genetic gains as well as eliminating the inbreeding depression through self pollination which may occur in open-pollinated seed orchard seed. It is estimated that the mass pollination of full-sib families may increase genetic gains in stem volume by up to 12% compared to an open pollination seed orchard containing the top 20 selected clones (Griffin *et al.* 1999). The deployment of full sib *E. globulus* families is likely to be advantageous in the short and medium term until developments in clonal propagation reduce the cost of clonal propagation.

These new pollination procedures have also opened the way for mass production of hybrid seed to allow either:

- (i) high selection intensities to be applied to identify elite genotypes which can also be efficiently cloned for deployment or

- (ii) direct deployment of elite hybrid families, thereby eliminating the constraint of clonal propagation all together.

A key element in the efficient production of hybrid seed is the ability to use *E. globulus* as the female parent due to the ease of controlled crossing. This is advantageous as selections are already established in orchards (eliminating the cost of grafting and establishing arboreta of minor species), controlled crossing is usually operationally easier using *E. globulus* as the female due to its large flower size, the seed set per capsule (29 seed per capsule under controlled crossing) is usually much higher than the smaller flowered species and the species has a great flowering window in Chile (August to December).

The most-likely solution to overcome the structural barrier to using *E. globulus* as a female in crosses with small-flowered species is to cut the *E. globulus* style, thereby reducing the distance that the pollen tube must grow to reach the base of the *E. globulus* style. Unpublished reports of early attempts to produce hybrids between *E. globulus* and *E. nitens* using this techniques were noted to be unsuccessful (Potts and Cauvin 1988; Gore *et al.* 1990). However, it is believed that in these cases, either the style was completely removed or pollinated several days after cutting. Bioforest data has shown that even intra-specific crossing is unsuccessful if the style is completely removed, but successful when up to 2/3 of the style is removed. Intra- and inter-specific hybrids have been produced using the cut style technique with females of smaller-flowered species such as *E. camaldulensis*, *E. grandis* and *E. dunnii* and *E. urophylla* (Harbard *et al.* 2000). Interspecific hybrids with females of *E. globulus* and pollen of the small-flowered *E. dunnii* have now been produced using the cut style technique in Australia (Barbour and Spencer 2000). In this case, leaving 60% of the style resulted in greater seed set than when 80% of the style was left.

3. RESEARCH PLAN

The proposed research will focus on six critical questions:

1. What is the optimal style length and flower stage to produce hybrids with *E. globulus* as a female?

Determine the optimal position and manner to cut the *E. globulus* style and the effect of flower maturation on the success of hybridisation for each species combination.

The development of efficient systems for the production and exploitation of interespecific hybrids of *Eucalyptus globulus*. Proposal for a higher degree by research project (2001-2003). University of Tasmania. Faculty of Science & Engineering. School of Plant Science.

FUNDACION PARA LA INNOVACIÓN AGRARIA

PROGRAMA DE FORMACIÓN PARA LA INNOVACIÓN AGRARIA

APOYO A LA PARTICIPACIÓN EN ACTIVIDADES DE FORMACIÓN

(VENTANILLA ABIERTA)

BID-FP-V-2002-1-F-57

MATERIAL ASOCIADO

2. Can the efficiency of controlled pollination procedures using females of the small-flowered species be improved?

Currently the production of hybrids with *E. globulus* necessitates the use of small flowered species as the female parent. The cut-style treatment has been reported as successful with several of these species (*E. camaldulensis*, *E. grandis* and *E. dunnii* and *E. urophylla*; Harbard *et al.* 2000). However, attempts to use this technique with the delicate style of *E. nitens* have to date been either unsuccessful (Williams *et al.* 1999) or resulted in very poor seed set (10% capsule harvest; 9 seed/100 flowers crossed; Harbard *et al.* 2000; Bioforest unpubl. data for capsule set), even with intra-specific crossing. This project will therefore explore other approaches to improving the pollination efficiency for *E. nitens* and possibly other small flowered species. For example, the development of new means of style or whole flower isolation has the potential to substantially increase pollination success in these small-flowered species. It has already been shown in *E. globulus* that the traditional bagging technique results in poorer capsule set compared with placing a tube over the style (e.g. *E. globulus* Williams *et al.* 1999; Harbard *et al.* 2000).

3. Are there reciprocal effects in F₁ hybrid success and performance?

There are increasing numbers of reports of reciprocal effects in the success and performance of hybrids in *Eucalyptus*. Reciprocal effects have been reported by Harbard *et al.* (2000a) in *E. dunnii* x *grandis* F₁ crosses. In a reciprocal 3x3 factorial, *E. grandis* females produced twice as many seed as *E. dunnii* females, however a significantly higher percentage of the F₁'s produced using the *E. dunnii* as female died. However there was no difference in the ratio of normal to defective seedlings. Sedgley and Granger (1996) reported ovule degeneration in crosses between *E. spathulata* and *E. platypus*, including failure of the hybrid zygote to divide. However when *E. spathulata* was the female, there was also slower embryo development and reduced cellularization of the endosperm. de Assis (2000) reports that in the majority of reciprocal crosses, rooting ability showed a maternal effect. When used as female, easy to root species or clones produced easier to root hybrids. Also it was noted that *E. dunnii* x *grandis* and *E. dunnii* x *urophylla* hybrids had fewer abnormalities than their reciprocals which suggested that there may be some type of maternal effect in their expression. Reciprocal effects have also been reported in *E. grandis* x *tereticornis* crosses for seed quality traits and seedling morphology by Bierwagen *et*

al. (2000) and for hybrid vigour in reciprocal crosses involving *E. urophylla*, *E. camaldulensis*, and *E. exserta* by Kha and Cuong (2000).

The success and performance of F_1 hybrids produced using *E. globulus* as a female will be compared with exactly the same crosses produced using the small-flowered species as the female parent.

4. Does style length or development stage affect the genetic composition of hybrid progenies?

There has already been reported loss of genetic material from one-or-other parent in interspecific backcrosses of *E. grandis* x *globulus* to either parent (Myburg et al. 2000). However this distortion was mainly in favour of the recurrent parent *E. grandis* which resulted in resistance to the introgression of donor DNA from *E. globulus* into the *E. grandis* genetic background. Similar effects have been reported in *E. gunnii* x *globulus* F_2 's (Vaillancourt et al. 1995). One explanation for these losses may involve differences in floral morphology and be due to linkage with genes affecting pollen tube growth. There are large differences in the pollen tube length between *E. globulus* and *E. nitens* and *E. gunnii*. Evidence would suggest that the style length and pollen tube length is inherited in an intermediate manner in the F_1 (Tilyard and Potts unpubl. data) but there is like to be segregation in pollen tube length. The smaller pollen tubes are likely to be linked with other genetic material from the smaller flowered species and thus it will be the more *E. globulus*-like genotypes that tend to effect fertilisation after growing through the full style. The frequency of genetic material from the small flowered species is likely to increase if the style length is reduced. If this is the case then the efficiency of backcrossing may be manipulated by changing style length to increase the frequency of genes of one or other species. This project will investigate the effects of the length of the *E. globulus* style cut and reciprocal effects on the genetic composition of backcross progeny.

There is also evidence that seed set per capsule is increased by style cutting at anthesis compared to normal pollination of the receptive stigma which raises the possibility that the receptive stigma may be discriminating against some pollen genotypes. If this were the case then cutting the style may alter the genetic composition of the resulting progeny. There is also the possibility that this trend may be accentuated by cutting the style of less mature flowers. This project will therefore use hybrid backcrossing as a tool to detect such treatment effects on the genetic composition of hybrid progenies. If such effects can be

detected in hybrid progeny then it may be worthwhile testing for more subtle effects in intra-specific crossing.

Large progeny sizes of *E. gunnii* x *globulus* and *E. nitens* x *globulus* backcross families will be used to test these effects. Differences between progenies will be examined using both quantitative (e.g. levels of abnormalities and growth, morphological differences) and molecular (isozymes or microsatellites) approaches.

5. Do *E. globulus* genotypes differ in their success of hybridisation?

There is increasing evidence that parents of the same species may differ markedly in their propensity of hybridisation. For example, Harbard et al (2000) report marked differences in the success of hybridisation amongst *E. dunnii* females. Volker (1995) also reports marked differences in the later success of hybridisation between *E. globulus* females of different provenance (Taranna vs King Island).

Variation in hybridisation potential amongst *E. globulus* females will be assessed using pollen parents of *E. gunnii*, *E. nitens* and *E. viminalis* as well as backcrosses involving *E. gunnii* x *globulus* and *E. nitens* x *globulus* pollen. Crossing will be undertaken using the cut style technique and progenies assessed for levels of abnormalities and early performance. Intra-specific crosses will also be undertaken as a control and the same females (up to 50 different genotypes) used for each pollen species combination to determine whether differences in hybridisation potential are species-specific.

6. Can hybrid performance be predicted by pure species performance?

Differentiation of hybrid breeding strategies depends on a knowledge of the genetic parameters of hybrid populations and how these relate to those estimated from the pure species populations. Key issues are whether the best pure species selections also produce the best hybrid combinations, their relative variability and the magnitude of specific combining effects in pure species and hybrid (Potts et al. 2000). The full-sib progeny generated from (5) will be established in field trials and genetic parameters (additive and non-additive effects) for early growth will be studied and the genetic correlations between cross types determined.

4. SIGNIFICANCE AND APPLICATION OF THE RESEARCH

The successful selection of elite hybrids for either cloning or deployment as full-sib families will initially depend upon having the capacity to produce and test large numbers of genotypes (Perrow and Cotterill 2000; Griffin *et al.* 2000). This project will result in the development of efficient procedures for the large scale production of F₁ hybrids for screening to allow a high selection pressure to be applied to identify elite hybrid combinations or specific genotypes.

While currently most research in forestry has focused on F₁ hybrids. In most cases, key traits of significance (e.g. frost resistance – Tibbits *et al.* 1991 and wood properties Tibbits *et al.* 1995) are often inherited in an intermediate manner in the F₁'s and it may be difficult to find exceptional combinations of key characteristics. This research will therefore explore the possibility of backcrossing to generate unique trait combinations. The identification of such genotypes will allow the favoured *E. globulus*-like characteristics to be extended into plantations on colder sites currently supporting *E. nitens* plantations and increase the economic worth of plantations on colder sites.

With the development of efficient pollination techniques and selection of appropriate female parents the large-scale production of selected F₁ or back-cross hybrid families by mass-controlled pollination may also be possible using grafted seed orchards, avoiding the reliance on clonal propagation for hybrid deployment. The cost of clonal propagation is seen as a major limitation to hybrid deployment in countries such as Australia.

References

- Barbour, L. (1997). Breeding better blue gums. *Landscape* 13, 37-41.
- Barbour, E.L. and Spencer, N. (2000). The potential of a crossing technique for interspecific hybridization between *E. globulus* and *E. dunnii*. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 390-394. (Department of Primary Industries: Brisbane.)
- Bierwagen, R., Sgarbi, F., Lanelli, C. and Amaral, W.A.N. (2000). Genotypic maternal influences on seed quality and seedling morphology of *E. grandis* × *E. tereticornis* hybrids. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 259-264. (Department of Primary Industries: Brisbane.)
- Cauvin, B. (1988). Pistil treatments for improved fertility in hybridization of *Eucalyptus gunnii* (Hook). In 'Sexual Reproduction in Higher Plants: Proceedings of the 10th International Symposium on the Sexual Reproduction of High Plants, 30th May-4th June 1988, University of Siena, Siena, Italy'. (Ed. P.G. M. Cresti, E. Pacini.) pp. 321-325. (Springer-Verlag: Berlin.)
- de Assis, T.F. (2000). Production and use of *Eucalyptus* hybrids for industrial purposes. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF

- Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 63-74. (Department of Primary Industries: Brisbane.)
- Ellis, M.F., Sedgley, M. and Gardner, J.A. (1991). Interspecific pollen-pistil interaction in *Eucalyptus* L'Her. (Myrtaceae): The effect of taxonomic distance. *Annals of Botany* 68, 185-194.
- Gore, P.L., Potts, B.M., Volker, P.W. and Megalos, J. (1990). Unilateral cross-incompatibility in *Eucalyptus* : the case of hybridisation between *E. globulus* and *E. nitens*. *Australian Journal of Botany* 38, 383-394.
- Griffin et al, 1999. Mass controlled pollination of *Eucalyptus globulus* : a practical reality. *Can. J. For. Res.* 29:1457-1463.
- Griffin, A.R., Harbard, J.L., Centurion, C. and Santini, P. (2000). Breeding *Eucalyptus grandis* × *globulus* and other inter-specific hybrids with high inviability - problem analysis and experience with Shell Forestry projects in Uruguay and Chile. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 1-13. (Department of Primary Industries: Brisbane.)
- Harbard, J.L., Griffin, A.R. and Espejo, J. (1999). Mass controlled pollination of *Eucalyptus globulus*: a practical reality. *Canadian Journal of Forest Research-Journal Canadien de la Recherche Forestiere* 29, 1457-1463.
- Harbard, J.L., Griffin, R. and Centurion, C. (2000a). Reciprocal hybrid crossing between *E. dunnii* (Maiden) × *E. grandis* (Hill ex Maiden). In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 435-439. (Department of Primary Industries: Brisbane.)
- Harbard, J.L., Griffin, R., Espejo, J.E., Centurion, C. and Russell, J. (2000). "One stop pollination" a new technology developed by Shell Forestry technology unit. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 430-434. (Department of Primary Industries: Brisbane.)
- Kha, L.D. and Cuong, N.V. (2000). Research on hybridisation of some *Eucalyptus* species in Vietnam. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 139-146. (Department of Primary Industries: Brisbane.)
- Myburg, A.A., Griffin, R.A.R., Sederoff, R.R. and Whetten, R. (2000). Genetic analysis of interspecific backcross families of a hybrid of *Eucalyptus grandis* and *Eucalyptus globulus*. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 462-466. (Department of Primary Industries: Brisbane.)
- Perrow (MacRae), S. and Cotterill, P. (2000). Breeding strategies for the next generation of clonal forests. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S. Dungey, M.J. Dieters and D.G. Nikles.) pp. 44-47. (Department of Primary Industries: Brisbane.)
- Potts, B.M. and Cauvin, B. (1988). Inbreeding and interspecific hybridisation of *Eucalyptus*. In 'International Forestry Conference for the Australian Bicentenary'. Albury-Wodonga, Australia. (The Australian Forest Development Institute.)
- Potts, B.M., Volker, P.W., Tilyard, P.A. and Joyce, K. (2000). The genetics of hybridisation in the temperate *Eucalyptus*. In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S.D. Dungey, M.J. and Nikles, D.G.) pp. 200-211. (Department of Primary Industries: Brisbane.)

- Rasmussen, G.F., Bower, D.L.M. and Borralho, N.M.G. (1995). Genetic control of micro-propagation characteristics in *Eucalyptus nitens* x *E. globulus* and implications for selection of clones. In "Eucalypt plantations: Improving Fibre Yield and Quality' Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb.'. (Eds B.M. Potts, N.M.G. Borralho, J.B. Ried, R.N. Cromer, W.N. Tibbits and C.A. Raymond.) pp. 289-292. Hobart, Tasmania. (CRC for Temperate Hardwood Forestry.)
- Peredo, 1999. Características y propiedades de la madera de *Eucalyptus*.
- Tibbits, W., Dean, G. and French, J. (1995). Relative pulping properties of *Eucalyptus nitens* x *E. globulus* F1 hybrids. In "Eucalypt plantations: Improving Fibre Yield and Quality' Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb.'. (Eds B.M. Potts, N.M.G. Borralho, J.B. Ried, R.N. Cromer, W.N. Tibbits and C.A. Raymond.) pp. 83-84. Hobart, Tasmania. (CRC for Temperate Hardwood Forestry.)
- Tibbits, W.N., Potts, B.M. and Savva, M.H. (1991). Inheritance of freezing resistance in interspecific F1 hybrids of *Eucalyptus*. *Theoretical and Applied Genetics*. 83, 126-135.
- Sedgely, M. and Granger, L. (1996). Embryology of *E. spathulata* and *E. platypus* (Myrtaceae) following selfing, crossing and reciprocal inter-specific pollination. *Australian Journal of Botany* 44, 661-671.
- Shelbourne 1999. Some insights on hybrids in forest tree improvement In 'Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium, 9-14th April 2000 Noosa, Queensland, Australia'. (Ed. H.S.D. Dungey, M.J. and Nikles, D.G.) pp. 200-211. (Department of Primary Industries: Brisbane.)
- Vaillancourt, R.E., Potts, B.M., Manson, A., Eldridge, T. and Reid, J.B. (1995). Using RAPDs to detect QTLs in an interspecific F2 hybrid of *Eucalyptus*. In "Eucalypt plantations: Improving Fibre Yield and Quality' Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb.'. (Eds B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond.) pp. 430-431. Hobart, Tasmania. (CRC for Temperate Hardwood Forestry.)
- Volker, P.W. (1995). Evaluation of *Eucalyptus nitens* x *globulus* for commercial forestry. In "Eucalypt plantations: Improving Fibre Yield and Quality' Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb.'. (Eds B.M. Potts, N.M.G. Borralho, J.B. Ried, R.N. Cromer, W.N. Tibbits and C.A. Raymond.) pp. 222-225. Hobart, Tasmania. (CRC for Temperate Hardwood Forestry.)
- Williams, D.R., Potts, B.M. and Black, P.G. (1999). A single visit pollination method for *Eucalyptus globulus*. *Australian Forestry* 62, 346-352.

5. DETAILS OF RESEARCH EXPERIENCE AND EXPERIENCE

1978-1979

Contract with INFOR (Chilean Forest Institute) in order to develop projects of forestry related with species and origins of arid and semiarid zones and. The project was financed by CONAF and the United Nations (PNUD).

1979-1983

Investigator of the Department of Forestry of INFOR. Main topics of investigation: research of exotic species introduction and origins. (*Pinus* and *Eucalyptus*).

1984

The development of efficient systems for the production and exploitation of interspecific hybrids of *Eucalyptus globulus*. Proposal for a higher degree by research project (2001-2003). University of Tasmania. Faculty of Science & Engineering. School of Plant Science.

Project Leader of the activity "Evaluation of species introduction of species in the arid and semiarid zone of Chile."- Program of Investigation: CONAF/ PNUD/ FAO/ CHI76003.

1985-1987

Project Leader of Project "Silviculture Management of Eucalyptus species" financed by INFOR/ CORFO and forests companies. The main responsibility was plan and implement silviculture techniques applied to fast growing species such as *E. globulus*, *E. nitens*, *E. regnans* *E. delegatensis*.

The program of investigation included several aspects like: nursery, soil preparation, nutrition, weed control. Other silvicultural traits were also evaluated like: spacings, thinnings, prunings and coppice management. This program began the investigation of genetic improvement of species of the Eucalyptus genus in Chile. The investigation included genetic variation of progenies and origins, selection of families and research on vegetative propagation through cuttings.

1988-1989

Student of the program of Post Graduate degree in the University of Sao Paulo in Piracicaba- Brazil. This program was financed through a scholarship of the International Development Research Center (IDRC- Canada). The main area of investigation of the program was guided to the forestry and the genetic improvement of Eucalyptus. The developed thesis included a comparison of several genetic materials in experimental plantations. The objective was to compare the propagation for seeds and clones of select families. The clones were proven on pure stands and also in arrangements. The main characteristics evaluated in the experimental plots were the basic density of the wood and the cubic volume of the trees for the production of pulp.

1990

Project Leader of the program of genetic improvement of INFOR. The main developed activities included the production of plants and the establishment of progeny trials of *E. globulus* and *E. nitens*.

1990-1994

Assisting Professor of the University of Concepción. Faculty of Forestry Sciences. The course of genetic forest improvement was designed. The work consisted of formal classes and the development of topics of investigation with the students, as well as the orientation of works of thesis with forests companies (Forestal Chile and Forestal Arauco).

1994

Technical Manager of CONAF (Corporación Nacional Forestal).
Strategic planning for the Forest Service of the State (1994-2000).

1990-1997

The development of efficient systems for the production and exploitation of interspecific hybrids of *Eucalyptus globulus*. Proposal for a higher degree by research project (2001-2003). University of Tasmania. Faculty of Science & Engineering. School of Plant Science.

* Project Leader of the program "Fibre Yield Improvement Program (FYIP) of the group Santa Fe developed by SHELL FORESTRY in Chile. The master plan considers the improvement of the yield and the quality of the pulp of the plantations of *Eucalyptus* of the company. The main goals achieved until 1997 were:

* Producing thousand of seedlings and clones in the nursery corresponding to hundred of families of open pollination for the commercial plantations and also for genetic test in the field.

* Designing and plant 70 genetic trials in the field: species and origins, hybrid test, clonal test, seedling and clonal seed orchards.

* Harvesting seed for the requirements of the nursery including the Seed Production Areas (SPA) and seed orchards (SSO, CSO).

* Crop of seed in commercial stands of *E. globulus* and *E. nitens* for sale.

* Developing a program of studies in technology of the wood including direct and indirect mensurations of the physical and chemical properties of the wood. Relationship with the industrial plant of Santa Fe.

* Beginning a program of investigation of reproductive biology of *E. globulus* and *E. nitens*. The main activities included phonology, pollen processing and storage in laboratory, controlled pollination controlled . The main hybrid synthesized have been *E. nitens* x *E. globulus*, *E. nitens* x *E. camaldulensis*, *E. nitens* x *E. viminalis*.

* Promoting genetic exchange (pollen and seeds) with other foreign companies like FOSA (Uruguay), SAPPI& HLH (Sudafrica), RIOCELL (Brazil).

* Manage an annual budget of U\$ 300.000.

* Collecting, manage and process a data base of several genetic trial called GENETICA call.

* Using the genetic generated information values of genetic improvement for the genetic purification of progeny trials. (seedlings and cuttings).

* Giving information to the clonal program clonal for the deployment of operational clones to the field.

1998

Scientist Research of Genetic division of the Bioforest company Corp.

➤ *Eucalyptus* Genes project.

A mass control-pollination of *E. globulus* spp *globulus* program based on one single visit was implemented in Arauco with a goal of 30 Kgs of seed during the period 1998-2001

- A research project called : "Reproductive biology and hybridization of *Eucalyptus* (FONTEC-CORFO)" is under development.

6. PUBLICATIONS

- 1-
- | | |
|-------------------|--|
| TITULO | Perspectivas del uso de la micropropagación en la silvicultura |
| FECHA | 1990 |
| AUTORES | Espejo, J.; Arce J.,P.; Rojas V.,P. |
| DATOS PUBLICACION | Santiago, Chile, Chile Forestal. 8p. |
| SERIE | Chile Forestal. Documento Técnico n.044 |
- 2.-
- | | |
|-------------------|--|
| TITULO | Nuevas especies para la forestación en Chile |
| FECHA | 1980 |
| AUTORES | Prado D.,J.A.; Barros A.,S.; Rojas V.,P.; Barros R.,D. |
| DATOS PUBLICACION | Santiago, Chile, CONAF. 8p. |
| SERIE | CONAF. Chile Forestal. Suplemento n.Jul/80 |
| NOTAS | Publicado como suplemento de Chile Forestal 5(58)80. |
- 3-
- | | |
|-------------------|---|
| TITULO | Metodología para la instalación y análisis de ensayo de introducción de especies forestales |
| FECHA | 1980 |
| AUTORES | Prado D.,J.A.; Barros A.,S.; Rojas V.,P.; Barros R.,D.; Vita, A.; Rustom, A.; Cogollor, G.; Alvarez, S. |
| DATOS PUBLICACION | Santiago, Chile, Proyecto CONAF/PNUD/FAO. 68p. |
| SERIE | CONAF/PNUD/FAO. Proyecto FO: DP/CHI/76/003. Documento de Trabajo n.31 |
| PROYECTO | Investigación y Desarrollo Forestal. Patrocinado por CONAF/PNUD/FAO. No.FO: DP/CHI/76/003 |
| NOTAS | Este Informe es resultado de un convenio con la Universidad de Chile y el INFOR.
En Btca. también Informes I y II (INFOR 127 e INFOR 128). |
- 4-
- | | |
|-------------------|---|
| TITULO | Metodología para la instalación y análisis de ensayo de introducción de especies forestales |
| FECHA | 1980 |
| AUTORES | Prado D.,J.A.; Barros A.,S.; Rojas V.,P.; Barros R.,D.; Vita, A.; Rustom, A.; Cogollor, G.; Alvarez, S. |
| DATOS PUBLICACION | Santiago, Chile, Proyecto CONAF/PNUD/FAO. 68p. |

The development of efficient systems for the production and exploitation of interespecific hybrids of *Eucalyptus globulus*. Proposal for a higher degree by research project (2001-2003). University of Tasmania. Faculty of Science & Engineering. School of Plant Science.

SERIE	CONAF/PNUD/FAO. Proyecto FO: DP/CHI/76/003. Documento de Trabajo n.31
PROYECTO	Investigación y Desarrollo Forestal. Patrocinado por CONAF/PNUD/FAO. No.FO: DP/CHI/76/003
NOTAS	Este Informe es resultado de un convenio con la Universidad de Chile y el INFOR. En Btca. también Informes I y II (INFOR 127 e INFOR 128).

5-

TITULO	Efecto de la preparación del sitio, fertilización y control de la competencia en el establecimiento de <i>Eucalyptus globulus</i>
FECHA	1986
AUTORES	Rojas V.,P.
DATOS PUBLICACION	Nacimiento, Chile, Forestal Río Vergara. 4p.
SERIE	Forestal Río Vergara . Avances en Investigación Forestal n.DID/09
NOTAS	Este artículo está basado en el trabajo de Prado D.,J.A.; Rojas V.,P.: "La importancia de la preparación del sitio y la fertilización en el establecimiento de plantaciones de <i>Eucalyptus globulus</i> en la zona semiárida de Chile". INFOR, 1986.

6-

TITULO	Crecimiento de algunas especies de los géneros <i>Cupressus</i> , <i>Eucalyptus</i> y <i>Pinus</i> en la costa de la Región del Maule, VII Región
FECHA	1979
AUTORES	Barros A.,S.; Rojas V.,P.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura; CORFO. 20p. maps, grafs, tpls.
SERIE	INFOR. Informe Técnico n.086
RESUMEN	Se evalúa la respuesta de esas especies, a través de los parámetros de supervivencia y desarrollo, para seleccionar aquellas que pueden ser incluídas en futuros planes de forestación

7-

TITULO	Los eucaliptos como alternativa para la forestación en la VIII Región
FECHA	1979
AUTORES	Prado D.,J.A.; Rojas V.,P.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura; CORFO. 27p. maps, grafs, tpls.
SERIE	INFOR. Informe Técnico n.089
RESUMEN	Se entregan resultados de supervivencia y desarrollo del

género *Eucalyptus* plantadas experimentalmente en el predio La Leonera, Comuna de Tomé

8-	
TITULO	Plantaciones experimentales con especies de <i>Eucalyptus</i> en la Zona Central, secano costero de la V Región
FECHA	1980
AUTORES	Rojas V.,P.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura; CORFO. 21p, anexos, grafs, tbls.
SERIE	INFOR. Informe Técnico n.092
RESUMEN	Se consideran dos lugares de ensayo (Peñuelas y Santa Marta), en los cuales se comparan las especies probadas, a los 10 años de edad. Los parámetros considerados en el análisis son: Mortalidad, DAP, altura y área basal

9-	
TITULO	Crecimiento de especies de los géneros <i>Pinus</i> y <i>Eucalyptus</i> . Secano costero. VI Región
FECHA	1980
AUTORES	Barros A.,S.; Rojas V.,P.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura; CORFO. 27p. tbls.
SERIE	INFOR. Informe Técnico n.093
RESUMEN	Los resultados son de parcelas experimentales instaladas durante los años 1967, 1969, 1970, en el predio San Antonio de Petrel, comuna de Pichilemu, a los 10 años de edad SECANO COSTERO / INTRODUCCION DE ESPECIES / CRECIMIENTO / CHILE / SEXTA REGION / SAN ANTONIO DE PETREL / PICHILEMU / <i>Eucalyptus delegatensis</i> / <i>Eucalyptus fastigata</i> / <i>Eucalyptus globulus</i> / <i>Eucalyptus grandis</i> / <i>Eucalyptus melliodora</i> / <i>Eucalyptus obliqua</i> / <i>Eucalyptus regnans</i> / <i>Pinus contorta</i> / <i>Pinus coulteri</i> / <i>Pinus jeffreyi</i> / <i>Pinus nigra</i> / <i>Pinus ponderosa</i> / <i>Pinus radiata</i>
MATERIAS	
UBICACION	SEDE SANTIAGO
SOLICITE POR	Chile. INFOR. Informe Técnico n.093

10-	
TITULO	Crecimiento de seis especies forestales ensayadas entre Arauco y Llanquihue
FECHA	1979
AUTORES	Rojas V.,P.; Barros R.,D.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura. 25p. map, tbls.
SERIE	INFOR. Informe Técnico n.094
RESUMEN	Resultados obtenidos, a los 10 años, con 6 especies (<i>Eucalyptus globulus</i> , <i>E. delegatensis</i> , <i>Pinus radiata</i> , <i>Pseudotsuga menziesii</i> , <i>Nothofagus obliqua</i> , <i>N. alpina</i>)

plantadas en parcelas experimentales en la zona Sur de Chile. La especie de mayor crecimiento fue *E. delegatensis*

11-

TITULO	Sistema computacional INTROESP (Introducción de Especies Forestales)
FECHA	1984
AUTORES	Rojas V.,P.; Salas G.,G.
EN	CONAF. Chile Forestal vol.10, n.108, pp. 22-23

12-

TITULO	Preparación del sitio y fertilización en el establecimiento de plantaciones de <i>Eucalyptus globulus</i> en la zona semiárida de Chile
FECHA	1987
AUTORES	Prado D.,J.A.; Rojas V.,P.
EN	INFOR. Ciencia e Investigación Forestal vol.01, n.1, pp. 017-027

13-

TITULO	Ensayos de procedencias de <i>Eucalyptus camaldulensis</i> Dehnh en la zona semiárida de Chile
FECHA	1987
AUTORES	Barros A.,S.; Rojas V.,P.
EN	INFOR. Ciencia e Investigación Forestal vol.01, n.1, pp. 029-040

14-

TITULO	Propagación vegetativa por estacas en <i>Eucalyptus camaldulensis</i> Dehnh
FECHA	1987
AUTORES	Rojas V.,P.; Arce J.,P.; Arriagada B.,M.
EN	INFOR. Ciencia e Investigación Forestal vol.01, n.2, pp. 001-009

15-

TITULO	Resultados preliminares en fertilización de apoyo para <i>Eucalyptus globulus</i>
FECHA	1988
AUTORES	Toral I.,M.; Rojas V.,P.
EN	INFOR. Ciencia e Investigación Forestal vol.02, n.3, pp. 045-056

16-

TITULO	Silvicultura clonal de <i>Eucalyptus</i>
FECHA	1990
AUTORES	Rojas V.,P.
EN	INFOR. Ciencia e Investigación Forestal vol.04, n.1, pp. 120-123

17-

The development of efficient systems for the production and exploitation of interespecific hybrids of *Eucalyptus globulus*. Proposal for a higher degree by research project (2001-2003). University of Tasmania. Faculty of Science & Engineering. School of Plant Science.

TITULO	Análisis del desarrollo del quillay (<i>Quillaja saponaria</i> Mol.) en la zona árida y semiárida chilena
FECHA	1983
AUTORES	Prado D.,J.A.; Barros A.,S.; Rojas V.,P.; Barros R.,D.
EN	Universidad de Chile. CEZA. Terra Arida n.04, pp. 438-454
CONGRESOS	Congreso Internacional de Estudios de Zonas Áridas y Semiáridas. La Serena, Chile, 15-19 Enero 1980. Patrocinado por Universidad de Chile

18-

TITULO	Seminarios presentados al Curso de Genética y Mejoramiento Forestal. Resúmenes. Abstracts. Primer semestre 1995
FECHA	1995
AUTORES	Rojas V.,P.
DATOS PUBLICACION	Concepción, Chile, Universidad de Concepción. Facultad de Ciencias Forestales. [83p.]

19-

TITULO	Estudio de las especies del género <i>Prosopis</i> en la Pampa del Tamarugal. Tomo I. Revisión bibliográfica y actualización de la información existente en el país
FECHA	1981
AUTORES	Wrann H.,J.; Prado D.,J.A.; Aguirre A.,J.J.; Rojas V.,P.; Barros R.,D.; Hernández C.,M.
DATOS PUBLICACION	Santiago, Chile, INFOR. 170p., anexos. tpls, 2 map.
NOTAS	Parte del Programa Pampa del Tamarugal, de CORFO.

20-

TITULO	Programa de conservación, ordenación y mejoramiento genético
FECHA	1994
AUTORES	Rojas V.,P.
EN	Fundación Chile; Grupo Silvícola; CONAF Diversificación y silvicultura. Nuevas experiencias. Tercer taller silvícola. Concepción, Chile, Octubre 1994. pp.103-145
CONGRESOS	Taller silvícola, 3. Concepción, Chile, Oct. 1994. Patrocinado por Fundación Chile; Grupo Silvícola; CONAF

21-

TITULO	Evaluación ensayos introducción de especies vegetales existentes en zonas silvestres áridas y semiáridas de Chile. Informe final
FECHA	1985
AUTORES	Rojas V.,P.; Vita A.,A.; Jobet J.,M.; Peralta P.,M.; Cabello M.,M.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura; Universidad de

The development of efficient systems for the production and exploitation of interspecific hybrids of *Eucalyptus globulus*. Proposal for a higher degree by research project (2001-2003). University of Tasmania. Faculty of Science & Engineering. School of Plant Science.

Patrocinado por	Chile. Facultad de Ciencias Agrarias, Veterinarias y Forestales. 141p, anexos, 1 map.
NOTAS	CONAF/PNUD/FAO. n.CHI-83-017 Informe preparado para el Proyecto CONAF/PNUD/FAO/CHI-83-017. Actividad II.1.
22-	
TITULO	Especies forestales exóticas de interés económico para Chile
FECHA	1986
AUTORES	Prado D.,J.A.; Barros A.,S.; Wrann H.,J.; Rojas V.,P.; Barros R.,D.; Aguirre A.,S.
DATOS PUBLICACION	Santiago, Chile, INFOR. División Silvicultura; CORFO. 168p. ilus, grafs, maps, tbls.
PROYECTO	Introducción de especies forestales. Patrocinado por CORFO
RESUMEN	Entrega los resultados más relevantes obtenidos en el programa de selección de especies introducidas, para que los forestadores cuenten con información respecto a especies que podrían reemplazar al género <i>Pinus</i> en replantación. Se consideran 3 zonas edafoclimáticas, sugiriendo áreas de forestación para especies principales: <i>Eucalyptus</i> , <i>Pinus radiata</i> , <i>P. muricata</i> y <i>Pseudotsuga menziesii</i>
23-	
TITULO	Evaluación de parcelas experimentales de introducción de especies
FECHA	1979
AUTORES	Barros R.,D.; Rojas V.,P.
DATOS PUBLICACION	Santiago, Chile, Universidad de Chile. Facultad de Ciencias Forestales. 127p.
TESIS	Universidad de Chile. Facultad de Ciencias Forestales. Grado de Ingeniero Forestal
24-	
TITULO	Efecto de la preparación del suelo, fertilización y control de la competencia en el establecimiento de plantaciones de <i>Eucalyptus globulus</i> en la zona semiárida de Chile
FECHA	1985
AUTORES	Prado D.,J.A.; Rojas V.,P.
EN	Webb, D., ed.; Barros A.,S., ed. Forestación en zonas áridas y semiáridas. Actas. [2º Encuentro Regional CIID, América Latina y El Caribe. Santiago, Chile. 13-17 May.1985] . pp.233-247
CONGRESOS	Encuentro Regional CIID, America Latina y El Caribe. 2. Santiago, Chile, 13 y 17 May.1985. Patrocinado por INFOR; CIID [Canada]

25-

TITULO	Sistema computacional para el archivo y procesamiento de informacion de ensayos de introducción de especies
FECHA	1985
AUTORES	Rojas V.,P. Webb, D., ed.; Barros A.,S., ed.Forestación en zonas áridas y semiáridas. Actas. [2º Encuentro Regional CIID, América Latina y El Caribe. Santiago, Chile. 13-17 May.1985] . pp.348-402
EN	Encuentro Regional CIID, America Latina y El Caribe. 2. Santiago, Chile, 13 y 17 May.1985. Patrocinado por INFOR; CIID [Canada]
CONGRESOS	

26-

TITULO	Situación actual de los programas de introducción de especies forestales en Chile. Informe I.
FECHA	1979
AUTORES	INFOR; Universidad de Chile
DATOS PUBLICACION	Santiago, Chile, INFOR. División Forestal; Universidad de Chile. Facultad de Ciencias Forestales. 386p, anexos.
Patrocinado por	CONAF/PNUD/FAO. n.CHI-76-003 Documento interno.
NOTAS	Participantes: Barros A.,S., Rojas V.,P., Barros R.,D., Navia A.,P., Vita A.,A., Toro V.,J., Cogollor H.,G.. Actividad I-2.2. Introducción de especies forestales. El Informe III se publicó como Documento de Trabajo n.31 del Proyecto. En Btca, también Informe II (INFOR 128).

27-

TITULO	Areas cubiertas por ensayos de especies y ubicación de nuevas experiencias. Informe II
FECHA	1979
AUTORES	INFOR; Universidad de Chile
DATOS PUBLICACION	Santiago, Chile, INFOR. División Forestal; Universidad de Chile. Facultad de Ciencias Forestales. 87p, apend. Cuad.
Patrocinado por	CONAF/PNUD/FAO. n.CHI-76-003 Documento interno.
NOTAS	Personal participante: Barros A.,S., Rojas V.,P., Barros R.,D., Navia A.,P., Prado D.,J.A., Elgueta S.,H., Vita A.,A., Toro V.,J., Cogollor H.,G., Caldentey P.,J. El Informe III se publicó como Documento de Trabajo n.31 del Proyecto. En Btca. también Informe I (INFOR 127).

28-

TITULO	Otras coníferas como alternativa para la forestación en la zona centro sur del país
FECHA	1981
AUTORES	Rojas V.,P.
EN	INFORSeminar: Nuevas especies forestales de interés económico para Chile , 27p.

29-

TITULO	Fibre Yield Improvement Program (FYIP) of <i>Eucalyptus globulus</i> Labill in Santa Fe Group, Chile. In:Proceedings IUFRO Conference on Silviculture and Improvement of <i>Eucalyptus</i> , Salvador Brasil
FECHA	1997
AUTORES	Rojas V.,P., Griffin A.R
EN	In:Proceedings IUFRO Conference on Silviculture and Improvement of <i>Eucalyptus</i> , Salvador Brasil. Vol 1 206-211

30-

TITULO	Avaliacao de plantios clonais e multiclonais em relacao a outros materiais geneticos de <i>E. grandis</i> no Estado de Sao Paulo
FECHA	1990
AUTORES	Rojas V.,P
EN	Tese de dissertacao. Ciencias Florestais. ESALQ/Universidade de Sao Paulo. Brasil