forest industry research cooperatives

CONTROL-POLLINATION WITHIN THE EUCAYLYTUS NITENS BREEDING POPULATION :

PART I – RECOMMENDATIONS FOR POLLEN HANDLING

R.M. McConnochie

Report No. 35 May 2000

forest research

CONTROL-POLLINATION WITHIN THE EUCAYLYTUS NITENS BREEDING POPULATION :

PART I – RECOMMENDATIONS FOR POLLEN HANDLING

R.M. McConnochie

Report No. 35 May 2000

CONTROL - POLLINATION WITHIN THE *EUCALYPTUS NITENS* BREEDING POPULATION: PART I - RECOMMENDATIONS FOR POLLEN HANDLING.

R.M. MCCONNOCHIE

INTRODUCTION

In New Zealand *Eucalyptus nitens* is the species most widely grown for the production of short fibre pulp and, with increasing interest, for solid wood end uses. It is one of three eucalypt species that have been the focus for hardwood genetic improvement with the first major introduction and testing of *E.nitens* individual families in 1978.

An enlarged breeding population for the advancement of *Eucalyptus nitens* was established in 1990 and comprised of 312 open-pollinated families. About half were native population and the others second generation selections. The results from the progeny tests of these families allowed selection of best families as parents for the next generation of the breeding population. The best individuals among these families were grafted for seed orchard establishment.

A clonal archive of the new *Eucalyptus nitens* breeding population was established with a total of 190 clones from the progeny test. In 1998, 67 clones were planted at Rangipo, and a further 123 clones were planted in 1999 at Waiouru, central North Island. Observations have shown that a combination of extended cold winters and dry summers promote the production of flowers and seed in *E.nitens*. Both archive sites were selected based on these climatic factors.

Mating of the breeding population parents in the clonal archive can be achieved either by control pollination or open-pollination. There are three benefits of control-pollination that can be readily identified.

1. Control-pollination amongst an 'elite' group of breeding population clones would maximise the gains in selected traits.

2. Crosses between genotypes with different flowering times can be achieved. The main *E.nitens* breeding population is made up of selections of provenances from central Victoria where there are three separated populations/provenances of *E.nitens*; Toorongo, Rubicon and MacAlister. The breeding population contains a mixture of selections from all of these provenance groups. The flowering time of *E.nitens* can vary between genotypes from different provenances. With open-pollination, crossing between these genotypes is prevented; control-pollination enables these crosses to be made.

3. Control-pollination will allow new genotypes to be integrated into the breeding population which can be broadened and enhanced by the inclusion of selections from other breeding programmes or provenances if desired. Pollen may be brought into New Zealand under ERMA regulations whereas introduction of genotypes through grafting scions is likely to be difficult or not permitted. New pollen introductions can be applied to the genotypes in the breeding archive.

However, in spite of the advantages of control-pollinated breeding, open-pollination within the archive will allow random inter pollination amongst the best parents in the breeding population and result in outcrossed families. Based on good estimates of breeding values in the progeny test, parents can be backwards selected.

The techniques for collecting, extracting and storing pollen have been reviewed in preparation for initiating a control-pollinated crossing programme to produce seed for the development of the next generation of the breeding population and other genetic evaluation.

THE EUCALYPT FLOWER

In New Zealand, *Eucalyptus nitens* generally flowers from November to February. The extended period of flowering requires pollen to be collected and stored for later application to produce a broad range of crosses. Some fresh pollen could be used in control-pollination where the flower from one clone can be used directly to pollinate another, but there will be limited opportunities for using this method.
Eucalypts are insect pollinated. The pollen is present on the anthers and is mature when the operculum is shed. The stigma does not become receptive until 5 days later and this impedes self-pollination. When flowers are exposed to wind and sun, pollen shed is complete in 3-4 days after antesis occurs. To protect against contamination when collecting pollen, the bud should be collected prior to anthesis when the operculum has developed a yellow/cream colour.

FLOWER BUD COLLECTION AND POLLEN EXTRACTION

Various methods for obtaining clean, viable pollen for control-pollination have been developed and reported. Some of these systems involve extensive filtration and drying onto paper strips. A far more simple method can be followed with *E.nitens* whose pollen is characteristically loose and non-sticky. Flowers must be collected before the operculum has shed to avoid any possible contamination.

On a branchlet there will be flowers at different stages of maturity. (Figure 1.) Some will have opened fully, while others will be still green buds with immature pollen. Remove and discard any open flowers. Trim the leaves by 50% to reduce transpiration. Place the branchlet in a jar of water and over the ensuing days the immature buds will ripen and can be collected and added to the dish. Buds that are yellow and beginning to shed their operculum can be collected. The anthers can be removed using a sharp scalpel or scissors and stored in a dish. Store the dishes in a desiccator on a base of silica gel. It is not essential to separate pure pollen from the anthers, or indeed from the bud cup. A vigorous shake in a capped vial is adequate to shed the pollen.

Where pollen from several different genotypes is being collected, ensure that the samples are kept separate, during field collection and while ripening in the laboratory. Either physically isolate the samples or cover with a fine voile to avoid contamination. During collection in the field and transport to the laboratory, keep the flower buds cool. An increase in temperature and relative humidity greatly reduces the viability of the pollen in both opened and unopened buds.

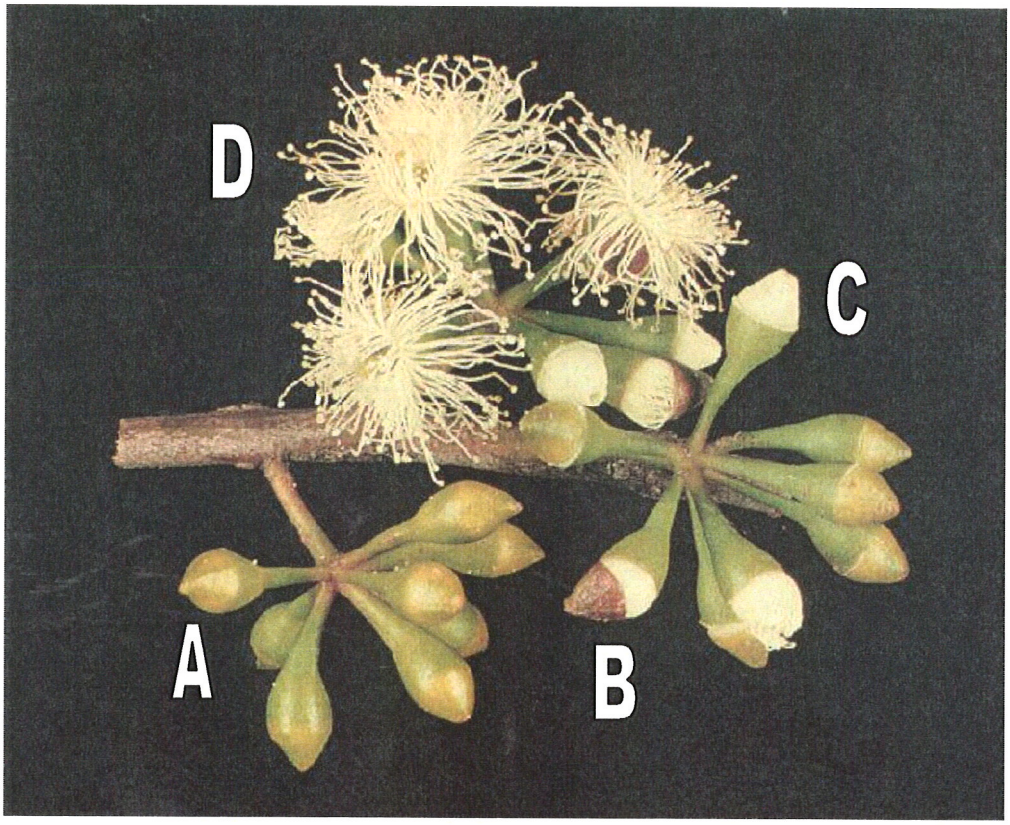


Figure 1. Flower development. (*E.viridis*) A. Inmature buds. B. Antesis (operculum shed). C. Post-anthesis. D. Fully open flower

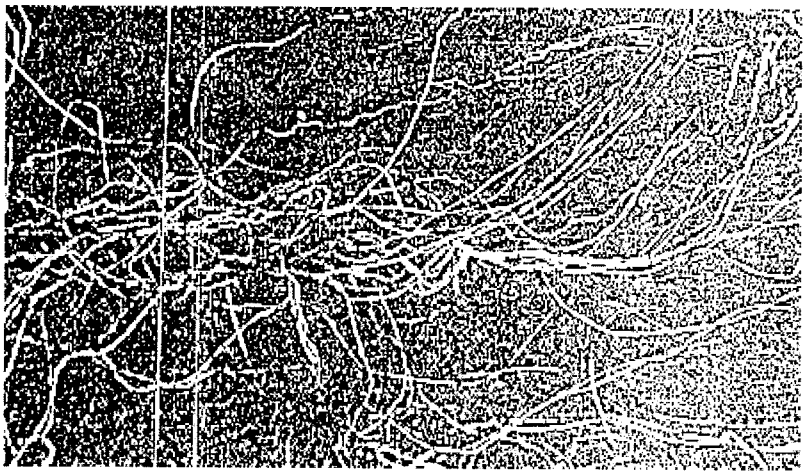
POLLEN VIABILITY TESTING

The testing of pollen viability before storage and /or before application is recommended. Tests are most commonly done *in vitro* using either liquid or a semi-solid agar media. There are minor variations in the media recipes that have been reported; but the following is a well recognised procedure.

To prepare the media, dissolve 150mg Boric Acid and 300g Sucrose in 1 litre distilled water (5gm of agar can be added to make a solid media). Store in small vials or plates and refrigerate until use.
Add a small amount of pollen and incubate at 20-30°C for 24 hours. Either place a drop of media onto a microscope slide or place the agar dish under a microscope, at 125X magnification. (Figure 2. Pollen tube growth). Count 100 grains, recording the number germinated or ungerminated and calculate the germination percentage.

It has been reported in many studies of pollen viability that there is a direct relationship between pollen germination and seed set. Pollen with germination of 10% can successfully produce seed but is much less effective than pollen with higher viabilities.

Figure 2. *E.nitens* pollen tube development



POLLEN STORAGE

Using fresh pollen for control-pollination is the most reliable option, but not always practical. Often pollen is required to be stored from one flowering season to the next. It is important for pollen to be promptly dried if it is to be stored for later use as the viability of pollen reduces significantly if it has not been dried. Once dried it can be kept for several weeks at room temperature, but is preferably left in a fridge (5°C) without reduction in viability. Place the flower buds in a desiccator with silica gel. Once the silica gel no longer changes from blue to pink the pollen is adequately dried for storage. Pollen is most successfully stored in a freezer at -18°C. (Boden 1958).

Recommended containers for pollen storage vary greatly and their choice is largely dependent upon availability from local suppliers. Pollen quantities are generally small (1-3mls) when compared to the litres of *Pinus radiata* pollen that are collected and stored for commercial control-pollination operations. The essential criteria for a storage vessel is that it is airtight and easy to access and handle during the field control–pollination process. Avoid storing large quantities in a single container that may be continuously removed and returned to cool storage throughout a pollination season.

VARIATION IN FLOWERING AND POLLEN VIABILITY

Within the breeding population the main flowering periods for individual clones and/or provenances may be quite separate so outcrossing between populations by open-pollination may be restricted. A study by Tibbits, 1989, characterised the variation in the flowering times of *E.nitens* between five different provenances. He found similarities in flowering time among trees within a provenance which indicated a strong genetic control of flowering at the population level.

In addition, the varying abundance of pollen among trees is not correlated with its ability to germinate. (Bolan, 1958). Therefore, pollen collected from trees producing even the smallest number of buds can yield pollen with good viability. Buds can therefore be collected from the grafted clones in the breeding archive even when only a few umbels are being produced. Variation in the viability of pollen within a single tree throughout the flowering period is not significant.

REFERENCES

Boden, R.W.(1958) Handling and storage of pollen in *Eucalyptus* breeding. Australian Forestry 22, 73-81.

Espejo, J., Ipinza, R., Potts, B.M. (1999). Manual de Cruzamientos Controlados para *Eucalyptus nitens* Dean et Maiden Maiden y *Eucalyptus globulus* (Labill).

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*. Austraslian Journal of Botany **38**, 383-394.

Gore P. and Potts, B.M. (1995). The genetic control of flowering time in *Eucalyptus globulus*, *E.nitens* and their F1 hybrids. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' pp. 241-242. Proc. CRCTHF-IUFRO Conf., Hobart.

Griffin.A.R, Ching.K.K, Johnson.K.W, Hand.F.C and Burgess.I.P. Processing *Eucalyptus* Pollen for use in controlled-pollination. Silvae Genetica 31,5-6 (1982)

Moncur.M.W. 1995. Techniques for Pollinating Eucalypts. ACIAR Technical Reports No.34.20p.

Potts, B.M; and Marsden-Smedley, J. B. (1989). *In vitro* germination of *eucalyptus* pollen: response to variation in boric acid and sucrose. Aust. J. Bot. 37, 429-41.

Potts,B.M and Gore,P.G. (2000). Reproductive biology and controlled pollination of *Eucalyptus*; A review. Symposium on hybrid breeding and genetics. Noosa, Australia

Tibbets, W.N. (1989). Controlled pollination studies with shining gum (*Eucalyptus nitens* (Dean and Maiden) Maiden). *Forestry* 62, 111-126.

Van Wyk.G. (1977) Pollen Handling, Controlled Pollination and Grafting of *Eucalyptus grandis*. South African Forestry Journal **101**, 47-53.