BY LIZ BARBOUR

Breeding better Blue Gums

Since the breeding of the Western Blue Gum, scientists at the Department of Conservation and Land Management have been working continually to improve its quality through artificial cross-pollination. The results so far have been better than expected.

For years, scientists at the Department of Conservation and Land Management (CALM) have been searching for the ideal plantation tree—one that will grow quickly and economically to produce high quality pulpwood for the paper industry. In 1980, attention focused on the Tasmanian bluegum (*Eucalyptus globulus*) and after planting trials at Busselton, Manjimup and Dwellingup showed promise, CALM embarked on a full-scale program to develop a new breed of tree to suit Western Australian conditions. The project was successful and the 'Western Blue Gum' was born. (See 'The Western Blue Gums are here', *LANDSCOPE*, Summer 1994-95).

CALM's research scientists are not resting on their laurels, however. They aim to refine further the genetic stock of their new tree in much the same way that agricultural scientists continually improve cattle and sheep breeds, by the controlled breeding of superior individuals.

In recent years our understanding of the science of genetics has progressed rapidly. Many of its new techniques and tools are extremely complex and 'high tech', such as those used in mapping the genes in plant and animal cells, while others are relatively simple such as the one described here, a new method for streamlining controlled pollination. Our new technique, simple and practical as it is, speeds up the discovery process by reducing costs and allowing for greater numbers of operations and replications to be undertaken. The breakthrough is that our controlled pollination method allows for every single flower on a superior tree to be utilised for seed production, however few flowers there might be. It is also quick and easy, and produces more seed per capsule than traditional methods.

The controlled pollination process is essential if scientists wish to achieve the greatest possible genetic gains from trees selected with a range of desired qualities, including greater height, straightness of bole or faster growth rates. It is carried out by manually fertilising the female parts of the flowers of one outstanding tree with the (male) pollen from another outstanding tree to produce seed with superior qualities of both parents. It is of the utmost importance that unwanted pollen from other trees is kept well away from the female flowers that are to be fertilised.

ARTIFICIAL POLLINATION—OLD STYLE

The classic artificial pollination method involves isolating a number of flowers on a whole branch of the tree. In the case of *E. globulus,* with its large flowers, this has proved to be inefficient and wasteful as only one or two flowers can be used on a branch of 10 or more. Previously opened flowers and buds still to open, have to be removed to prevent their pollen contaminating the 'ripe' flowers that are to be artificially pollinated. A 'ripe' flower is recognised when the operculum or bud cap separates from the base of the bud and can be lifted off with the fingers.

Previous page
Western Blue Gum seedlings growing in the CALM Manjimup nursery.

Below: A plantation of mature bluegums. The Western Blue Gums will produce more uniform trees, with greater wood volume.
Photos – Marie Lochman

Right: Open flowers of *Eucalyptus globulus* with the yellow anthers attracting the insect pollinators.

Below right: Buds and control-pollinated flowers at various stages in the process. Daily visits to a flowering tree are needed to ensure all flowers are used for control pollination.

Far right: The technician for the Genetic Deployment Unit, Nic Spencer, in the accelerated orchard, control-pollinating the flowers.
Photos – Liz Barbour

Below: A young plantation of blue gums.
Photo – Jiri Lochman

The technique involves first the careful removal of the pollen, contained in anthers of each of the 'ripe' flowers. Following this, the exposed female parts of the flowers, the styles, are washed with water to remove any contaminating pollen. The whole branch is then isolated by encasing it in a wire frame covered by an isolation bag, sealed tightly at both ends. The frame keeps the bag material from damaging the styles, while the isolation bag material is designed to stop insect entry but allow air and heat exchange. This ensures the environmental conditions inside the bag are similar to those experienced by flowers in the open. After four to seven days, depending on weather, the bag is opened at one end and the pollen from the selected 'father' tree is applied to each female stigma using a matchstick. The stigmas are recognised as receptive when they turn red and exude a sticky substance which will stimulate pollen germination. The bag is then resealed for a week or two while the fertilisation process takes place and is then removed to allow the maturation of the seed capsules.

ARTIFICIAL POLLINATION—NEW STYLE

The new single flower artificial pollination method was developed for the 'accelerated orchard'. Created of grafted plants from the best Western Blue Gum selections, this seed orchard is managed to produce earlier flowering and accelerated seed production. The grafts are potted to restrict root growth as this promotes earlier flowering than normal. In addition, a growth-retardant chemical, paclobutrazol, is applied to trees to stunt their growth, which has the added effect of stimulating flowering. The trees are purposely kept stunted to allow scientists easy access to the flowers for controlled pollination work. However, a side-effect of stunting is a reduction in the number of flowers as the trees' food supplies cannot support large flower numbers. As the pace of breeding and genetic deployment programs is determined by the number of crosses made and the amount of seed produced by controlled pollination, it was necessary to find a pollination method in which every flower could be used.

A number of methods to isolate single flowers were tried. Bagging the individual

flowers with various materials was time-consuming and it was difficult to stop the bag material from rubbing against the styles and stigmas and damaging them. In Tasmania, tree-breeders tried gluing straws over the style, but this time-consuming process also met limited success. CALM staff developed the idea of using a very small plastic tube to protect the style and stigma from unwanted pollen contamination and found this method to have the greatest success.

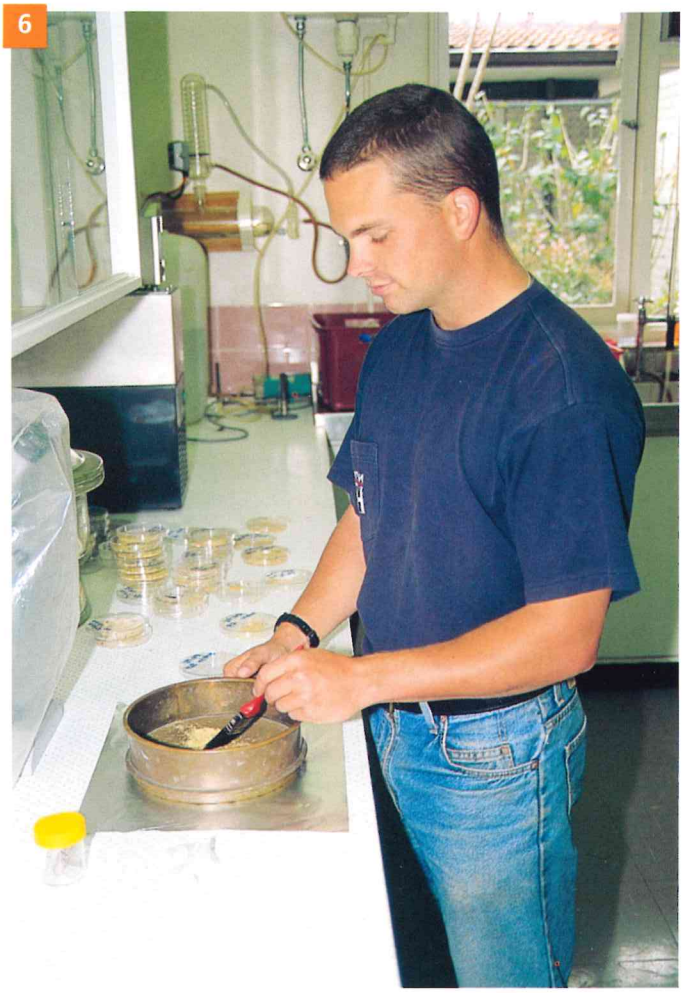
The method is simple. Every flower can be used as it becomes ripe. The bud cap is removed, the anthers are cut and collected for pollen extraction and the exposed style and stigma are washed to remove any contaminating pollen. A plastic tube, pinched closed at the apex, is then placed snugly over the style. These tubes need to be available in different sizes as style shape can vary between different Western Blue Gum clones. Each pollination is identified by a number scratched into a soft aluminium tag tied to the branch just below the flower and the details are recorded in a computer database system.

After a day or two when the stigma is receptive, the tube is removed and selected pollen is applied. The tube is then replaced. It appears to hold the pollen at the stigma surface, an advantage as the pollination program is carried out in winter, when wind and rain are more common. To indicate flower pollination the aluminium tag is folded in half. When the fertilisation process has taken place and the capsule is swelling in size, the style shrivels and falls off together with the tube. The seed capsule remains with the tag to be collected nine months later.

The success of this artificial pollination process is not only determined by the method but also by the quality of the pollen applied. Pollen will fall from the anthers within 24 hours of the flower opening. To ensure that majority of pollen is collected, the anthers are collected from the flowers in a sterile petri dish as the flowers are cleaned for control pollination. In the laboratory the anthers are dried in a desiccator to reduce or remove any risk of fungal contamination. Drying also makes the pollen easier to separate from the anthers through a sieve. The clean pollen can then be stored in small air-tight vials and checked for its viability.

The method used to check pollen viability involves assembling sterile plates which contain a solidifying agent together with a sucrose and boron mix. The sucrose-boron mix mimics the exudate produced by the receptive stigma and stimulates the pollen to germinate. Viable pollen will germinate after 24 hours incubation and can be observed under a scanning electron microscope.

We have produced as many as 92 seeds from a single capsule using the single flower isolation method and a vigorous pollen. However, this case was exceptional. On average, the single flower controlled pollination method produces 25 seeds per capsule compared with the average of 10 seeds per capsule produced by open pollination. These figures are low as they include flower abortion, which is a problem with *E. globulus*. Some clones suffer this abortion problem more than others and, in a few cases, it has been found that they can only be used to provide pollen in a crossing program.



The new single flower control pollination method. *1*) The cap is removed from the bud with the fingers. *2*) A scalpel blade is used to carefully remove the male anthers to expose the female style. *3*) The pollen on the anthers is collected in a petri dish for further processing. *4*) The style is washed with water to remove any contaminating pollen. *5*) The style is covered with a tube to isolate it from any contaminating pollen. This tube is removed when the known pollen is applied and the tube replaced whilst the pollination process occurs. *6*) The anthers are dried in the laboratory and then sieved to separate the pollen from the debris. *7*) The sieved pollen is collected on the tin foil, brushed together and stored in a small, labelled tube. Once this pollen has been tested for its viability it will be used to pollinate flowers on the trees.
Photos – Liz Barbour

The rapid development of the *E. globulus* plantation program in the State's south-west to approximately 15 000 hectares per annum has placed huge demands on CALM's tree-breeding program. Although producing seed by controlled pollination is expensive, CALM is committed to using this process as it is one of the most important steps in accelerating both the breeding and genetic deployment program.

It is critical, however, that the method is a precise exercise. The pollen needs to be pure and viable and there cannot be possible contamination in the controlled pollinator process. Accurate records of the process also need to be kept, particularly the percentage of the extracted seed. Using the new single flower method, only about five control pollinations are needed per cross on a wide selection of trees to move the breeding program into the next stage. This process allows CALM to maintain a closely spaced, stunted orchard right on the doorstep of its Como offices in Perth, allowing for easy access for intensive work during the flowering months. The simple technique of single flower control pollination will help us meet the demands of a growing industry.

THE FUTURE

Australia is not alone in recognising the outstanding economic potential of *E. globulus*. It has become one of the most sought-after species for pulpwood production, particularly in Portugal, Spain and Chile, where climates are ideally suited for its growth. By the end of the year 1995, 1.7 million hectares of *E. globulus* had been planted worldwide, 350 000 hectares of which had been planted in the five years between 1991 and 1995. By 1999 world plantings are expected to total 1.87 million hectares. A similar rate of growth of plantation expansion is already taking place in Western Australia. It can be confidently predicted that CALM's genetically superior strains of Western Blue Gum will be in demand not only in our own State, but also in an ever-expanding international market.

Liz Barbour is a research scientist and the manager of CALM's Forest Resources Services, Genetic Deployment Division. Her work has centred on the development of genetically superior Western Blue Gum strains and the single-flower pollination method. She can be contacted at CALM's Operational Headquarters in Como on (08) 9334 0302 or email lizb@calm.wa.gov.au.

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