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Techniques for Pollinating Eucalypts

M.W. Moncur

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Techniques for Pollinating Eucalypts

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Improving planting stock for use in commercial eucalypt plantations is a continuous process. Initially, open pollinated seed is collected from natural stands. Resulting progeny are evaluated for various traits, such as fast early growth or disease resistance. To further upgrade the population, controlled crosses may be made to concentrate the best alleles from a range of selected trees (Plate 1). Through this process of genetic improvement, plantations can be tailored by breeding for vigour and desirable end products, such as high wood density, high pulp yield, optimum fibre length, low extractives content, and a higher content of essential oils. This report describes the practical steps necessary to produce controlled cross seed of eucalypts.

Plate 1. Plantation of genetically improved *E. urophylla* in China.

Eucalypt flowers are bisexual, containing both male and female parts on the same flower (Plate 2). Individual flowers are protandrous, meaning pollen is shed before the stigma is receptive and thus self-pollination is avoided. In many eucalypt species, the trees produce many flowers over a long period in any given season of flowering (Plates 3 and 4). Therefore, there is ample opportunity, for selfing within the crown as flowers on individual trees develop at different times (Plates 4 and 5).

To produce a controlled cross, pollen (Plate 6) from anthers (Plate 7) of a selected male parent is placed on the stigma (Plate 8) of a female parent. The stigma must have been isolated from all other pollen sources. Pollen germinates on the stigma, grows down the style to the ovary and fertilises the ovules (Plate 9).

The timing of pollen shed from the anthers, and the time which then elapses until the stigma is receptive differ between species. The later may be as short as 1–2 days, or as long as 7–10 days. This variation is not surprising considering there are over 800 species of eucalypts adapted to a very large range of environmental conditions. It is essential that close attention be paid to the developmental pattern of the flower, so the correct stage of development can be identified, for both pollen collection and emasculation of flowers.

Selected parents may not flower at the same time. This is especially so when hybridisation is being attempted between different species; thus often pollen will have to be collected in advance and stored so that it is available.

When attempting pollen transfer between species (hybridisation) it is essential to understand the barriers that might inhibit success. For example, there is a varying degree of self-incompatibility between species of various subgeneric groups. Barriers also exist in the different flower structures. Pollen from flowers that

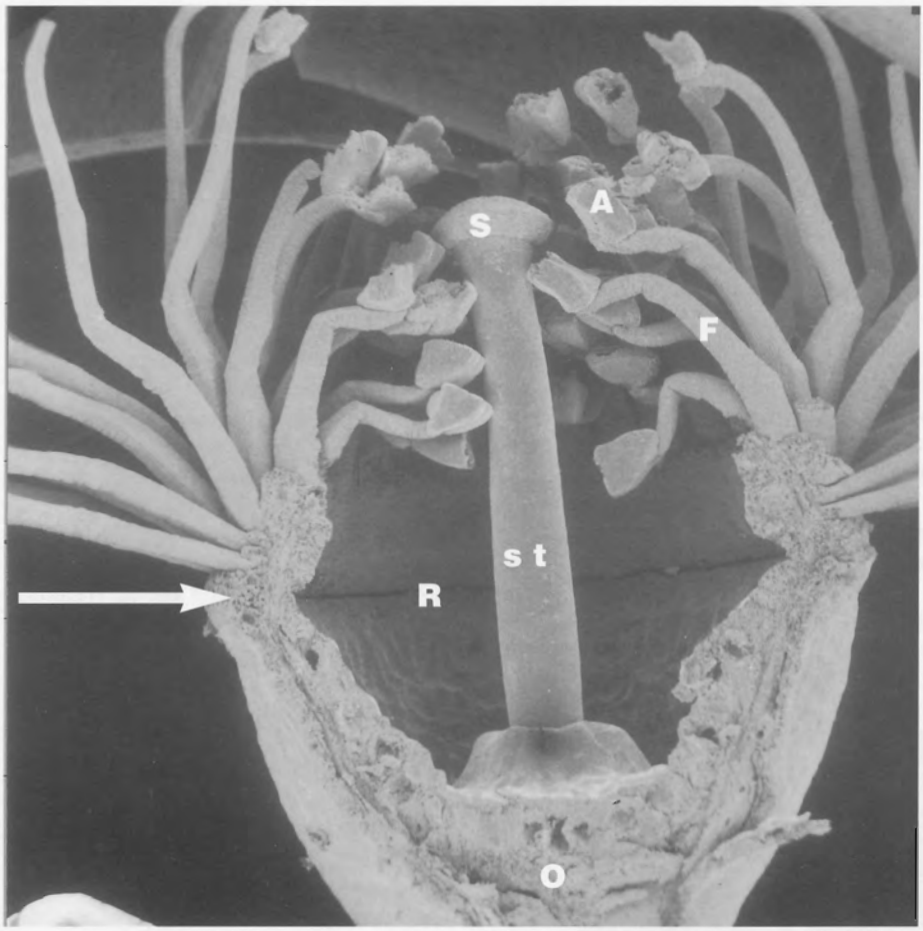

Plate 2. Flower of *E. melliodora*. ×25. A, anther. F, filament. S, stigma. ST, style. O, ovary. R, receptacle. Arrow indicates position of cut for emasculation.

have short styles will fail to fertilise flowers with long styles. As an example, pollen from *E. globulus* (long style) will be able to fertilise flowers of *E. nitens* (short styles) but not the reverse.

Pollen Collection and Storage

The timing of pollen shed in relation to floral processes varies between species. In some species such as *E. melliodora*, anthers dehisce while still enclosed by the operculum (cap) and pollen is shed into the receptacle. In other species, such as *E. camaldulensis*, the filaments may be almost fully expanded before the anthers shed their pollen.

To ensure there is no contamination from other pollen, flower-buds must be collected just before the operculum is shed. A change in operculum colour is a useful indicator that shedding of the operculum is imminent. Place the flower-buds on a piece of paper or a flat dish in an air-tight container such as a desiccator (Plate 10). The base of the container should be filled with silica gel to assist the drying process. As the flower-buds dry out (which usually takes from 1 to 3 days) the opercula fall off and the pollen is shed. It may be necessary to gently rub individual flowers over a course mesh of approximately 1–3 mm apertures to dislodge anthers. Flower-buds and shed material are

Plate 3. Tree of *E. globulus* in full flower.

Plate 4. Flowers of *E. gummifera*. Note range of stages.

then sieved to remove the pollen. In large-flowered species such as *E. camaldulensis,* flowers need only to be tapped gently for abundant pollen to be released onto a sheet of aluminium foil. Pollen is placed in a glass bottle or vial (Plate 11), sealed, labelled and stored at or below 0°C. The sieve needs to be cleaned between flower samples.

Pollen, for cross-pollinations should be as fresh as possible. It can be stored for a few days under low humidity in the desiccator, but viability drops considerably after 10 days of storage especially if it is not well dried. Long-term storage has been successful at temperatures of 0°C or below.

If there are insufficient flower-buds at the correct stage, cut off small branches, strip and discard opened flowers, place the branches in a bucket of water, remove most of the foliage to reduce desiccation, cover with a plastic bag and leave overnight in the laboratory. Next morning, flower-buds at the correct stage can be harvested and placed in the drying containers. Using this method, flower-buds can be harvested over a period of 3 to 5 days.

Testing Pollen Viability

Pollen viability should be tested as soon as possible after extraction and before storage. Testing should also be undertaken prior to pollen being used for pollinating flowers, especially if it has been held in storage for some months. Adequate fertilisation can sometimes be obtained using pollen with germination as low as 10%, but best results are obtained with higher viabilities. There are numerous methods of testing pollen viability. The method below is simple and has given consistent results.

Use a small screw-capped glass vial that has been sterilised. Prepare a medium of 30% sucrose, 150 ppm of boric acid and distilled water. Place 3 to 4 drops of the medium (sufficient to cover the base) into the vial. Add a small amount of pollen and place the vial in a germination cabinet at room temperature of 20–25°C.

Plate 5. Flowers of *E. maculata* at different stages of development.

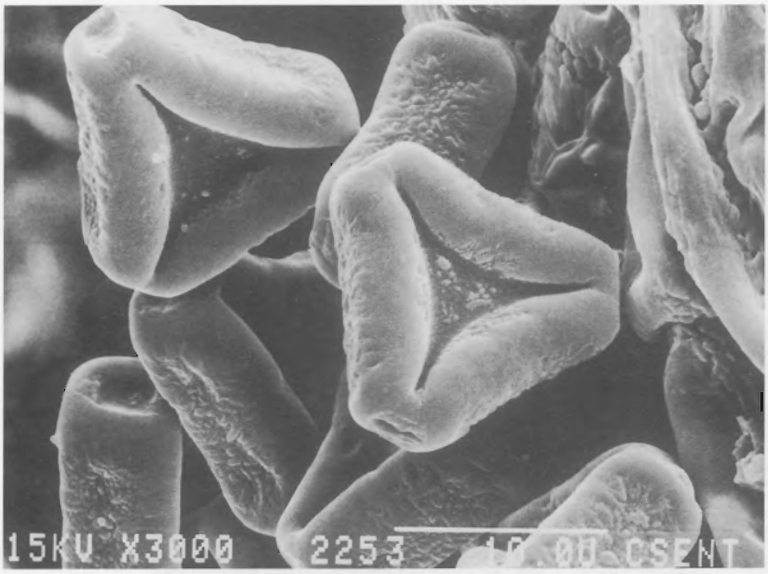

Plate 6. Pollen grains of *E. nitens*. ×5000 Scanning electron micrograph.

Plate 7. *E. grandis* anther shedding pollen. ×300 Scanning electron micrograph.

Plate 8. Pollen on stigma of *E. grandis*. ×100 Scanning electron micrograph.

Plate 9. Ovary and ovules of *E. grandis*. ×200 Scanning electron micrograph.

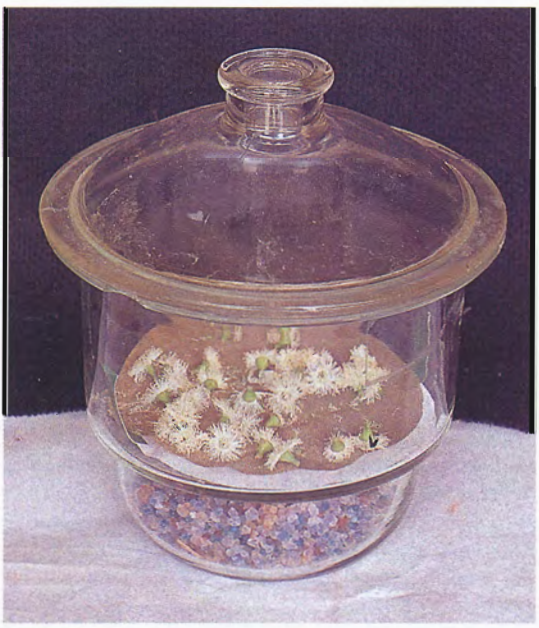

Plate 10. Flowers drying in desiccator. Note silica gel in base.

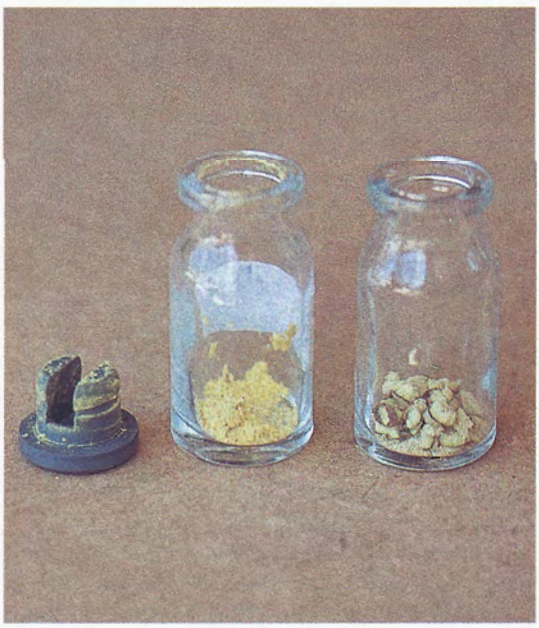

Plate 11. Pollen sieved into vial for storage at or below 0°C.

After germination, which usually takes 1 or 2 days, take the vial and using a 1 ml pipette extract a drop of liquid containing both germination medium and pollen. Place a drop on a microscope slide and view at about ×160 magnification. Germinated and ungerminated pollen can be counted and a germination percentage calculated.

Sequence of Pollen Collection

Step 1. Collect flower-buds that show operculum scar.
Step 2. Place in air-tight container with silica gel to dry.
Step 3. Shake and sieve. Collect pollen in vial.
Step 4. Seal and label vial. Store at or below 0° C.
Step 5. Test pollen viability.

Selection of Trees and Branches

The selection of trees and branches on which flowers will be pollinated is an important step. Firstly, the tree must be healthy (there is no point in spending a lot of time and effort on pollinations if the tree aborts its flower-buds or worse, dies). Secondly, chose a tree with a medium to heavy crop of flower-buds as this will give greater opportunities for selection of good quality flowers. Thirdly, choose unshaded branches where growth is vigorous. Branches low in the crown or close to the ground are unlikely to be the best.

Branches need to be strong as they will be supporting a pollination bag for approximately 14 days (depending upon species). Strong winds can break the branch because of the added weight of the bag. In some cases the bag may have to be tied to a nearby branch for support. Too much branch movement may result in the stigma coming in contact with twigs and leaves, and being damaged. Select a branch which has about 20 flower-buds at the correct stage for emasculation.

Emasculation and Isolation

The ideal time for emasculation is when the operculum has changed colour from green to a light green or whitish/yellow or reddish/brown as in *E. maculata* (see Plate 4). A good test of readiness is whether the operculum will flick off easily with the thumb nail. Emasculation at an earlier stage will result in a high level of abortion. At this stage the operculum scar (Plate 12) may be visible, although in subgenus Monocalyptus the scar may be difficult to distinguish from other tissue. In this subgenus extra care is needed to pinpoint the position of the operculum join as flower-buds will abort if cut too low on the operculum (Plate 2).

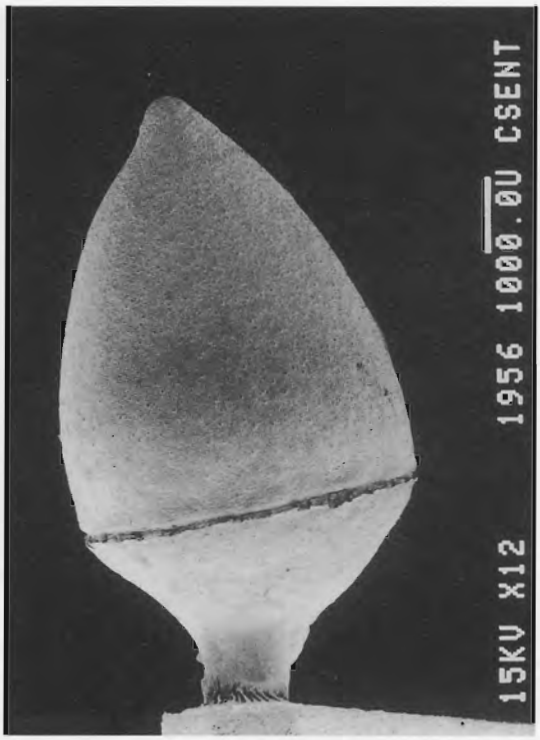

Plate 12. Operculum scar on *E. grandis* flower-bud. ×25 Scanning electron micrograph.

Using an emasculation tool (electrician's wire strippers ground down to produce a suitable cutting tip) (Plate 13) or a curved scalpel blade (Plate 14) cut through the tissue slightly below the operculum scar. Try to cut through the operculum and filaments in one circular cut so they come off in one piece (Plate 15). The base of the style can be damaged if the cut is too deep. Like-wise care should be taken when removing the cut portion not to damage the style and stigma. This is especially important with species where the flower-buds have pointed opercula, for example, *E. nitens.*

Once flower-buds have been emasculated (Plate 16) they must be isolated from other pollen. The usual method is to cover the branch and emasculated flower-buds with a protective bag (Plate 17). Before placing the branch inside the bag it may be necessary to remove some leaves to avoid a build-up of moisture inside the bag. However, be careful not to remove leaves subtending the inflorescences as this will reduce carbohydrate supply. If flower-buds are far

Plate 13. Emasculating tool.

Plate 14. Cut just below operculum scar with curved scalpel. Note colour of operculum.

Plate 15. Cut completed. Avoid damage to stigma when removing cut portion.

Plate 16. Emasculated flower.

Plate 17. Polyester pollination bags. Note different sizes.

enough back from the end of the branch they can be covered with a sleeve and no foliage needs to be removed. The bags can be turned into sleeves by cutting off the end with scissors.

Once emasculation is completed, cotton wool is wrapped around the base of the branch and the bag is closed and tied onto the wool. Cotton wool stops entry of ants and other insects that may carry pollen from other sources and contaminate the stigma. Next, the branch has to be clearly labelled, preferably with a metal tag and a generous piece of flagging tape. Remember the tag will be on the tree for an extended period. A percentage of labels do degrade and fall off, especially in windy conditions. It is recommended that two labels be used per bag. This small investment of extra time provides insurance for a process that has cost a lot of effort.

Pollination Bags

Commonly-used bags are made of polyester, casein or glassine.

Polyester bags are made from non-woven polyester material which allows the passage of heat and moisture, and has good light penetration. The material is water-resistant and does not rot. Polyester bags are washable and can be used repeatedly. Bags are available in a range of sizes, and each bag usually has an observation window.

Sausage bags (casein) allow passage of air and water but not as efficiently as polyester. Thus problems with excessive heat inside the bag can occur in hot weather. Sausage bags are mainly used on species with solitary flowers, for example, *E. globulus* as it is a waste of material to use one large polyester bag to cover only a few flowers. Casein is not rigid and needs a wire frame to keep it away from the stigma (Plate 18).

Paper bags (glassine) are made from translucent white material. Their reflective property ensures the air inside the bag remains cooler than the sausage bags. Paper bags deteriorate in wet conditions and can be used only once.

Applying Pollen

After 3–7 days, depending upon species, remove the bag and inspect the stigma. When receptive, the usually swollen stigma will be shiny, moist and sticky. If not, record the date of observation and replace the bag.

Plate 18. Sausage bag. Note wire support.

Once the stigma is receptive, pollen can be applied. This can be done by using a small brush (Plate 19), tooth pick, small stick or the lid of the vial (Plate 20). This procedure is repeated again in 2–3 days. Sometimes a third application may be necessary if there is doubt about stigma receptivity on a previous visit. It is very important that you rinse your hands and instruments with alcohol between pollinations to ensure no contamination occurs from pollen previously used. It is best to dedicate one brush per cross to avoid any chance of contamination.

Bag Removal and Seed Collection

Bags must be left in place until fertilisation is complete. When fertilisation is completed the style usually turns a pinkish-red colour and the stigma dries out. Once the stigma has dried there is no further chance of fertilisation, so remove the pollination bag. Delay increases the chance of stress to the developing flower-buds by high temperature and/or wind damage.

Depending upon species, it may take at least 12 months before the seed is ready for harvest. To assess seed maturity, collect 3 to 4 capsules (not the crosses) from the tree when they first show external signs of approaching maturity (usually a colour change from green to brown). The sample capsules can be allowed to dry out and open naturally, or cut open to check the colour of the seed. If the seed is a pale yellow-straw colour it is immature. Fully-mature seed will be brown or dark-brown, or in some species black. The danger of harvesting too early is that initial seed viability will be low and will decrease rapidly in storage.

Record Keeping

It is important to have a record of pollination details. Remember, the pollinated flower-buds may be on the tree for 12 months before the seed is mature. Data to be recorded should include male and female parent, date of emasculation, date of pollen application and date of bag removal (Plate 21).

Plate 19. Pollen transfer with small brush.

Plate 20. Pollen transfer with vial cap.

Plate 21. Controlled pollination record.

Control Pollination Records

Species .... Location .... Year ....

Bagged by .... Pollinated by.................................... Bags removed by ....

Capsules......................

Bag No.	Date	Female Parent	Male Parent Pollen	No. flower-buds	Date	Date Pollinated	No. flowers	Date Pollinated	No. flowers	Date Pollinated	No. flowers	Date Pollinated	No. flowers	Date Debagged	No. Capsules	Date Inspected	No. Capsules	Date Inspected	No. Capsules	Date Collected	No. Capsules

Sequence of Pollination (see Plate 22)

Step 1. Select branch. Remove old capsules, flowers and immature flower-buds. Remove excessive leaf material. Tag branch with metal tag.
Step 2. Emasculate flower-buds.
Step 3. Place pollination bag over branch.
Step 4. Inspect every 2–3 days.
Step 5. When the stigma is receptive apply pollen with brush or vial cap. Replace bag. Record event.
Step 6. Repeat pollen transfer after 2–3 days.
Step 7. Once fertilisation is complete, remove pollination bag.
Step 8. Collect mature capsules and check data on tag.

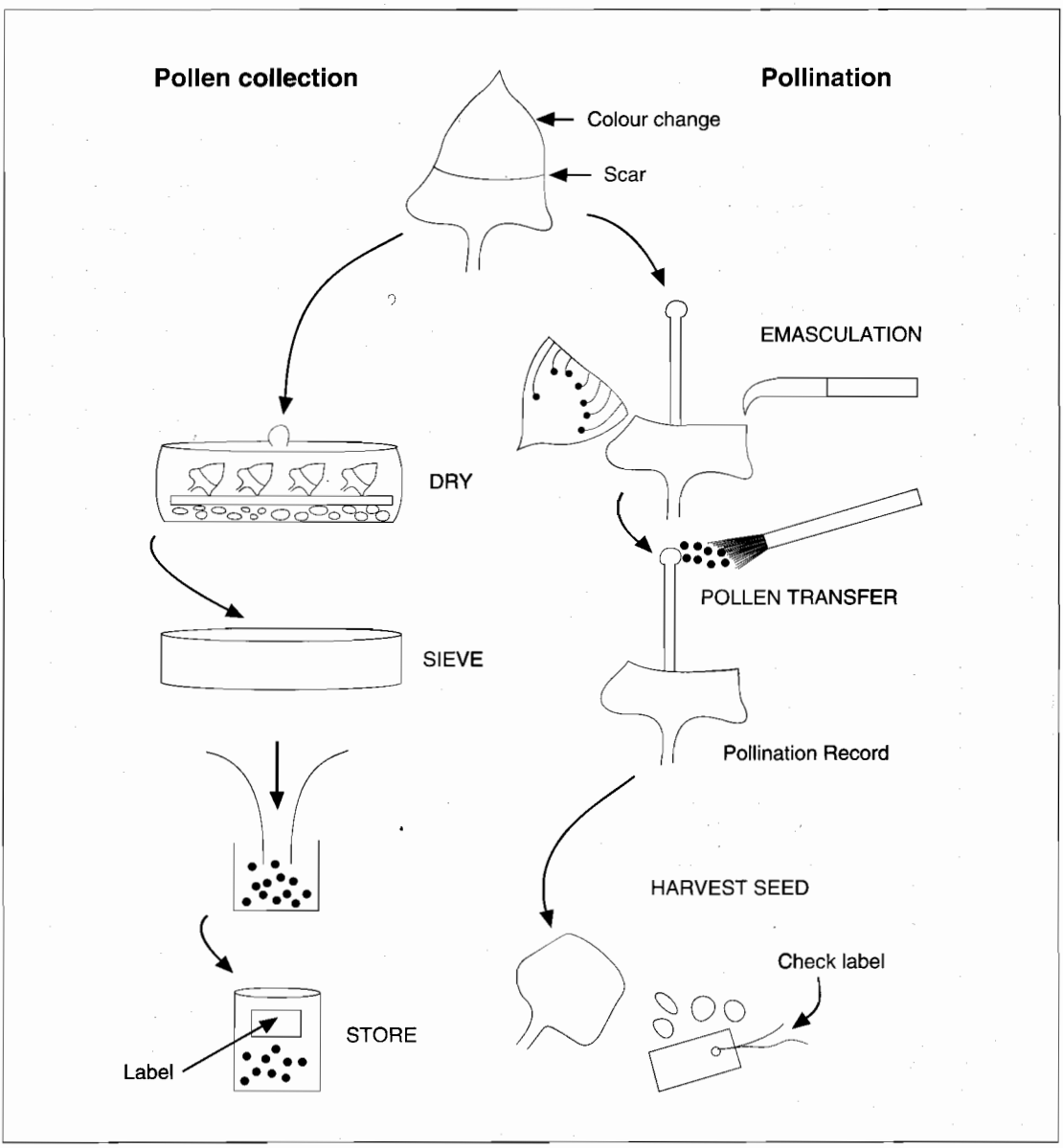

Plate 22. Techniques for pollen collection, emasculation and pollen transfer for controlled pollinations in eucalypts.

Seed Production

Cross-pollinations are usually carried out on trees growing in natural stands, or in established seed orchards, or breeding arboreta where trees may be at least 10 m tall. As eucalypt flowers form on the periphery of the canopy, their pollination is a difficult task without scaffolding, ladders (Plate 23) or travel towers (Plate 24).

Plate 23. Using a ladder to reach *E. camaldulensis* flowers.

Recently, intensively managed seed production systems have been developed which keep the height of the trees (usually grafts of selected phenotypes) to 2–3 m above ground level, for example, pollarded or as espaliers (Plate 25). Thus cross-pollinations can be carried out from the ground, increasing the numbers of flowers pollinated and operational efficiency. Heavy

Plate 24. Inspecting *E. globulus* flowers with a travel tower.

crops of flower-buds (Plate 26) and capsules (Plate 27) have been successfully produced from this system. To obtain small quantities of seed from a single cross it is possible to grow grafted material in small containers (Plate 28). These plants can be induced to flower within 12 months by applications of growth retardants such as paclobutrazol. Cross-pollinations can be successfully carried out on these potted plants.

Materials

The materials required for pollinating eucalypts are listed below (see Plate 29).

Pollination bag	Secateurs	Emasculation tool
Cotton wool	Scalpel	Small brush
Label	Scissors	Pollination record
Ties	Tweezers	Pencil
Vial	Eye piece	

Plate 25. Espalier orchard of 6-year-old grafted *E. nitens*.

Plate 26. Heavy bud crop on *E. nitens* following application of paclobutrazol.

Plate 27. Heavy capsule crop on *E. bicostata.*

Plate 28. Flower-buds on potted 18-month-old *E. nitens* treated with growth retardants.

Not shown on Plate 29, desiccator (Plate 10) and rope for tying back branches.

Pollinating bags - Polyester and glassine bags can be obtained from:

Duraweld Plant Breeding Supplies, Slater Rd, Eastfield Industrial Estate, Scarborough, North Yorkshire, Y011 34Z, England.

Diatex, 16 Chein de Saint Goban, F-67910, Saint-Fons, France.

Sullivan Company, 250 South Van Ness, San Fransisco, California 94103, USA.

Further Reading

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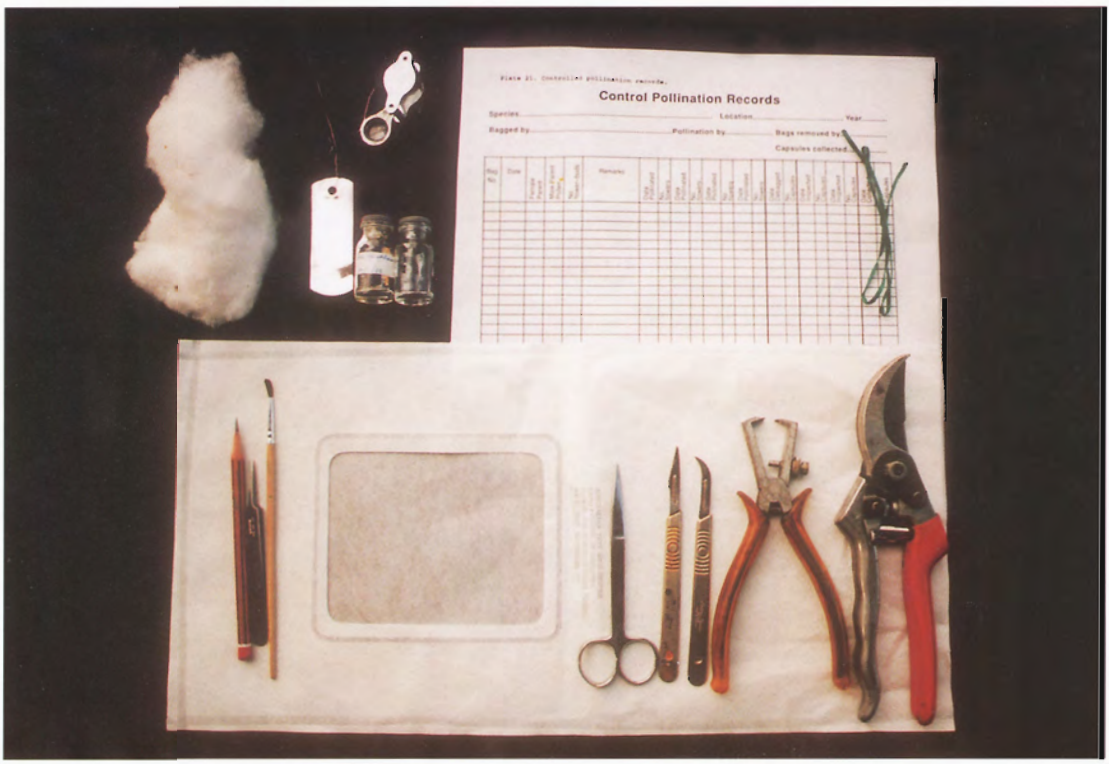

Plate 29. Materials used for controlled pollination.

Acknowledgments

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