

Figure 12. Insect (honey bee) carrying pollen on its body

Drying pollen over silica gel in a desiccator

- Prepare the flower buds for sieving by drying over silica gel in a desiccator for 3 days at room temperature.
- The base of the desiccator should be filled with fresh silica gel to assist the drying process.
- The plant material is then passed through a 45 micron sieve to remove the pollen.
- Pollen is placed in a glass bottle or vial, sealed, labelled and stored at sub-freezing temperatures (e.g. -18°C). Sub-freezing storage is best combined with a desiccant to maintain low humidity.
- Between flower sampling, all equipment must be cleaned by washing or spraying with alcohol.



Figure 13. Flowers drying in a desiccator

Drying pollen using a freeze-dryer

If available, a freeze-dryer (Figure 14) is a useful method of drying a large number of pollen samples quickly. The method of use is as follows, though operators should also familiarise themselves with the machine and read the operating procedures:

- mature flowers should be collected and loosely packed into glass vials (Figure 15)
- the vials are then placed in the specific chamber with rubber stoppers positioned for later sealing (Figure 16)
- freeze-dry the pollen for about 24 hours
- seal vials and store as described above.



Figure 14. A freeze-dryer



Figure 15. Collecting flowers for pollen extraction

Pollen for cross-pollination should be as fresh as possible. It can be stored for a few days under low humidity in desiccators, but its viability may drop considerably after 10 days of storage.

Melaleuca pollen has been successfully stored for short periods at temperatures of 3–5°C. For long-term storage, however, temperatures of –18°C to –20°C are recommended. Pollen of *M. alternifolia*, for example, will retain acceptable viability for 11 months if stored at –18°C in sealed vials (Figures 16 and 17).



Figure 16. Flowers in vials in freeze-dryer

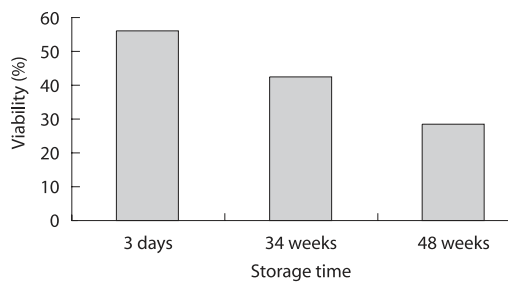


Figure 17. Decline in viability of pollen of *Melaleuca alternifolia* over time when stored in sealed vials at –18°C



Figure 18. Germinated pollen (×20 magnification) of *Melaleuca alternifolia*

Testing pollen viability

A pollen viability test should be carried out as soon as possible after collection of flower buds and extraction, before the pollen is stored. Testing should also be undertaken before the pollen is used for pollinating flowers, especially if it has been held in storage for some months. Adequate fertilisation can sometimes be obtained using pollen with a germination rate 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 consisting of 30% (300 g) sucrose and 150 ppm (150 mg) of boric acid in 1 litre of distilled water. Place 3–4 drops of the medium (sufficient to cover the base) into the vial. Add a small amount of pollen mix using a toothpick. Secure cap, shake and label the vials, and place them in a germination cabinet at 20–25°C for 24 hours.

After germination, which usually takes 1–2 days, use a 1 mL pipette to extract from the vial a drop of liquid containing medium and pollen. Place a drop on a microscope slide and view at about ×20 magnification. Germinated (Figure 18) and ungerminated pollen can be counted and a germination percentage calculated.

Selection of trees and branches for pollination

The selection of trees and branches on which flowers will be pollinated is an important step. Consider the following factors:

- Trees must be healthy. There is no point in spending a lot of time and effort on pollination if the tree aborts its flower buds because of stress; or worse, dies.
- Trees with a medium to heavy crop of flower buds should be chosen, as this will give greater opportunities for selection of quality flowers. In cases where trees must also serve as pollen parents, an initial survey and ranking of individuals based on the number of branches suitable for emasculation (removal of male flower parts) and as a pollen source aids selection.
- Select unshaded, strong branches where growth is vigorous. Branches low in the crown or close to the ground run the risk of damage by passing traffic.

Branches need to be strong, as they will be supporting a pollination bag for about 14 days. Strong winds may 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 or the pollination bag and being damaged.

- Be sure to replicate each cross on each mother tree as security against breakage. The replicates (bags) of each cross should be spread around the crown for added security.

Emasculation and isolation

A branch that has about 10 inflorescences, each subtended by a healthy vegetative bud, is selected. Inflorescences without a healthy vegetative bud at their apex may fail to grow, inducing capsule abortion. Any advanced, open flowers are removed. The aim is to emasculate about 50 flowers in each bag. Any immature capsules from last year's crop should also be removed at this point. To ensure there is no contamination from outside pollen, a pollination bag should be fitted to cover the inflorescences. Cotton wool or foam should be used at the base of the bag as insulation for the branch and to ensure exclusion of insects.

Removal of all male parts can be carried out with a small pair of tweezers. It is very difficult to emasculate the small flowers before they open. As anthers do not shed their pollen for a few days after extension, it is easier to emasculate when the staminal columns have started to uncurl. Advanced buds (Figure 19)



Figure 19. Advanced bud and freshly opened flowers which are used for emasculatation

can be emasculated by prying open the top of the bud with sharp tweezers (Figure 20) and carefully removing sepals and filaments so as not to damage the style, which is still bent at this stage (Figure 21).

Close examination with a $\times 10$ magnifying glass will show if anthers have been successfully removed. It may be necessary to remove some foliage to avoid a build-up of moisture within the bag.

Once emasculatation is completed, the pollination bag should be replaced and tied on (Figure 22). Next, the branch has to be clearly labelled, preferably with a metal tag or 'Dymo' tape. It must be considered that the tag will need to remain firmly attached on the branch for about 14 months.

For various reasons, some tags do fall off. It is thus suggested that two labels per bag be used (Figure 23). This small investment of extra time provides insurance for a process that has cost a lot of effort.

As a high level of outcrossing rate has been identified, it may be possible to pollinate without first emasculating each flower. This would save considerable time and place less stress on individual flowers. Pollination without emasculatation has been field tested and is suitable where some (<10%) self-pollination is acceptable.

Applying pollen

Before flower opening, the styles are small and bent over. After flower opening, the style straightens and increases in length. The stigma, on the tip of the style, enlarges and appears shiny, moist and sticky, indicating receptivity. Once the stigma is receptive,



Figure 20. Emasculating flowers of *Melaleuca alternifolia* using tweezers

pollen can be applied. Pollen is applied using a small brush (Figure 24) or stick, or the lid of the vial.

This procedure should be repeated during the subsequent few days, to ensure all stigmas receive pollen at the receptive stage. It is important to rinse hands and pollen applicator with ethanol between pollinations to ensure no contamination occurs from pollen used in a previous cross. It is recommended that one brush be used for each type of pollen, and labelled as such, to avoid any confusion of pollen source.

Sequence of pollination

- Step 1.** Select branch. Remove mature capsules, opened flowers and immature flower buds (Figure 25).
- Step 2.** Label branch with metal and colour tags (Figure 26). If a range of colours is available,

use of a colour specific to the pollen parent aids identification for subsequent pollinations.

- Step 3.** Emasculate flower buds (Figure 27).
- Step 4.** Place pollination bag over branch (Figure 27). Record event.
- Step 5.** Inspect after 2–3 days.
- Step 6.** When the stigma is receptive, apply pollen with brush or vial cap. Replace bag, record event (Figure 28).
- Step 7.** Repeat pollination after 2–3 days.
- Step 8.** Once fertilisation is complete, stigmas turn brown and abscise. About 14 days after last pollination, pollination bag should be removed (Figure 29).
- Step 9.** Collect mature capsules identified by their labels. This occurs at 14–18 months after pollination.



Figure 21. Bent styles after emasculation



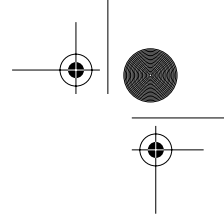
Figure 23. Colour tag and metal tag for bag labelling



Figure 22. Bagging the emasculated flowers



Figure 24. Pollinating using a paint brush



Baskorwati, L. 2006. Controlled pollination methods for *Melaleuca alternifolia* (Maiden & Betche) Cheel. Canberra, ACIAR Technical Reports No. 63.



Figure 25. Removing opened flowers and immature and mature capsules



Figure 26. Labelling the branch



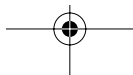
Figure 27. Emasculating the flowers of *Melaleuca alternifolia*; completed branches (inflorescences) are bagged



Figure 28. Applying pollen to receptive stigmas



Figure 29. Removing the pollination bag 14 days after pollination



Baskorwati, L. 2006. Controlled pollination methods for *Melaleuca alternifolia* (Maiden & Betche) Cheel. Canberra, ACIAR Technical Reports No. 63.



Figure 30. Fruit (capsules) of *Melaleuca alternifolia* at different stages of development: a. 2 weeks after anthers shed; b. 4 months old; c. 14 months old and ready to harvest. Capsules may be retained on a tree for some years.

Bag removal and seed collection

Pollination bags must be left in place until fertilisation is complete. When fertilisation is completed, the stigma is no longer sticky and shiny and has turned brown. This occurs about 7 days after pollination, at which time the pollination bag should be removed. Delay increases the chances of wind damage and/or stress to the developing flower buds by high temperatures.

In *M. alternifolia* it takes about 14–18 months for the seed to mature and be ready for harvest. Each mature capsule, which is dark brown in colour (stage C, Figure 30), consists of 20–25 seeds on opening.

Record keeping

It is important to keep a record of pollination details. Data to be recorded should include male and female parent, date of emasculation, date of pollen application and number of flowers pollinated at each treatment, and date of bag removal. Using a special Control Pollination Records sheet (Appendix 1) facilitates this process.

Materials

A list of the materials required for pollinating melaleucas follows. The materials are shown in Figure 31.

- magnifying headset
- magnifying glass
- labels (metal + wet-strength paper of various colours)
- ethanol
- metal ties + wire
- pollination record sheets
- small paint brushes
- pencil

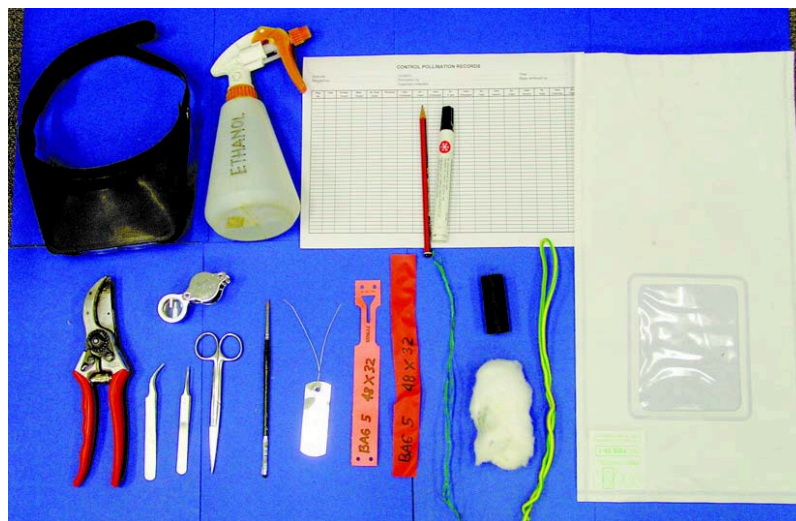


Figure 31. Materials used in controlled pollination



Baskorowati, L. 2006. Controlled pollination methods for *Melaleuca alternifolia* (Maiden & Betche) Cheel. Canberra, ACIAR Technical Reports No. 63.

- scissors
- waterproof marker
- tweezers
- pollination bags
- rope
- secateurs
- cotton wool or foam
- ladders (if required).

Acknowledgments

This technical report describes methods used in my PhD research. I am very grateful to ACIAR, ANU and CSIRO for institutional support. I am indebted to John Doran (CSIRO), Mike Moncur, Paul Warburton (CSIRO), Garry Baker (NSW, DPI) and Mauro Davanzo (ANU) for introduction to the technique and field assistance, and to Roger Heady and Cheng Huang (RSBS, ANU) for scanning electron microscopy. I am also grateful to my supervisory panel—Peter Kanowski, John Doran, Mike Moncur and Saul Cunningham—for comments and discussion during the preparation of this report.

References

- Brophy, J.J. and Doran, J.C. 1996. Essential oils of tropical *Asteromyrtus*, *Callistemon* and *Melaleuca* species. Canberra, ACIAR Monographs, No. 40.
- Butcher, P.A., Bell, J.C. and Moran, G.F. 1992. Patterns of genetic diversity and nature of the breeding system in *Melaleuca alternifolia* (Myrtaceae). Australian Journal of Botany, 40, 365–375.
- Craven, K.A. and Lepschi, B.J. 1999. Enumeration of the species and intraspecific taxa of *Melaleuca* (Myrtaceae) occurring in Australia and Tasmania. Australian Systematic Botany, 12, 819–927.
- Davis, R.L. 2003. The Australian tea tree industry. Paper presented at IFEAT international conference on Australia and New Zealand: essential oils and aroma chemicals — production and markets, Sydney. London, IFEAT. [CD-ROM]
- Doran, J.C. 1999. Cajuput oil. In: Southwell, I. and Lowe, R., ed., Tea tree: the genus *Melaleuca*. Medicinal and aromatic plants: industrial profiles vol. 9. Australia, Harwood Academic Publishers.
- Southwell, I. 2003. Tea tree: crop and productivity improvement. Paper presented at IFEAT international conference on Australia and New Zealand: essential oils and aroma chemicals — production and markets, Sydney. London, IFEAT. [CD-ROM]
- Doran, J.C., Baker, G.R. and Southwell, I.A. 2002. Improving Australian tea tree through selection and breeding. Canberra, Rural Industries Research and Development Corporation, RIRDC Publication No. 07/017.
- Hendrati, R.L., Baskorowati, L. and Kartikawati, N. 2002. Reproductive biology of *Melaleuca cajuputi* ssp. *cajuputi*. Proceedings of an international seminar on advances in genetic improvement of tropical tree species, Jogjakarta, Indonesia. Jogjakarta, CFBTI.
- Moncur, M.W. 1995. Techniques for pollinating eucalypts. Canberra, ACIAR Technical Reports, No. 34, 19p.
- Sedgley, M., Harbard, J. and Smith, R.M. 1992. Hybridisation techniques for acacias. Canberra, ACIAR Technical Reports, No. 20, 11p.

Further reading

