The Australian Centre for International Agricultural Research (ACIAR) was established in June 1982 by an Act of the Australian Parliament. Its mandate is to help identify agricultural problems in developing countries and to commission collaborative research between Australia and developing country researchers in fields where Australia has a special research competence.

Where trade names are used this constitutes neither endorsement of nor discrimination against any product by the Centre.

ACIAR TECHNICAL REPORTS SERIES

This series of publications contains technical information resulting from ACIAR-supported programs, projects, and workshops (for which proceedings are not being published), reports on Centre-supported fact-finding studies, or reports on other useful topics resulting from ACIAR activities. Publications in the series are distributed internationally to a selected audience.

Sedgley, M., Harbard, J., and Smith, R.-M. 1992. Hybridisation techniques for acacias. ACIAR Technical Reports No. 20, 11p.

ISBN 1 86320 060 6

Typeset and laid out by Arawang Information Bureau Pty Ltd, Canberra, Australia.

Printed by: Goanna Print Pty Ltd, Canberra, Australia.

Cover: Bagged spike-bearing branches of *Acacia auriculiformis*. The spikes at the bottom right are at the correct stage for bagging.

Hybridisation Techniques for Acacias

Margaret Sedgley, Jane Harbard and Rose-Marie Smith

Australian Centre for International Agricultural Research
Canberra 1992

Foreword

The genus *Acacia* includes over 1200 species, many of which have characteristics useful for industrial and community forestry. There are widespread plantings of acacias in Africa and Asia and, in recent years, attempts have been made to increase their productivity through selection and breeding. Controlled pollination has presented particular problems as the flowers are very small and are grouped into globular or cylindrical infloresences.

In Malaysia it was observed that some natural hybrids of *Acacia mangium* and *A. auriculiformis* were very vigorous and had desirable stem form characteristics for timber production. To capitalise on the hybrid vigour and other benefits of breeding, it was essential to develop simple, practical techniques to transfer pollen from the male parent to the stigma of the female parent.

In response to this problem, ACIAR commissioned a project which included the development of procedures for controlled crossing of acacias. The results of the work on methods by staff at the Waite Agricultural Research Institute in South Australia are reported in this ACIAR Technical Report. It is intended that they will provide a guide to enable tree breeders to take advantage of the benefits of hybrids or other germplasm derived from the controlled crossing of acacias.

G.H.L. Rothschild
Director, ACIAR

Hybridisation Techniques for Acacias

Margaret Sedgley,* Jane Harbard* and Rose-Marie Smith*†

MANIPULATION of the genetic composition of a plant via crossing and selection offers the opportunity for improvements in both yield and quality. Successful plant breeding relies on exploitation of the breeding system of the crop to achieve sufficient numbers of plants of the desired genetic combination for selection. The first stage of the process is the crossing of the selected parents by controlled pollination at the most fertile stage of both the female and male organs. Pollen collection and transfer to the female parent are the major stages, after which the progeny from the successful crosses are grown and selected for the desired characteristics inherited from one or both parents. Acacias present particular problems in hybridisation, as the flowers are very small and are grouped into spherical or cylindrical inflorescences, and methods developed for genera with large flowers, which are easy to cross, must be adapted. Hybridisation methods depend upon manipulation of the flower (Plate 1), for the transfer of pollen from the anther of the male parent (Plate 2) to the stigma of the female parent (Plate 3). Acacias produce compound pollen grains or polyads (Plate 4), which must germinate and produce pollen tubes to grow down the style (Plate 5) and penetrate the ovules (Plate 6), so that pod set (Plate 7) and seed and pod maturation can occur (Plates 8 & 9). This manual will address general aspects of hybridisation procedure, and illustrate how this has been developed for the controlled crossing of acacias.

Pollen Collection

It is important that the pollen used for hybridisation is of the correct species or cultivar, is not contaminated with stray pollen, pests or dirt, and has the ability to effect fertilisation and seed set. Flowers are generally isolated using pollination bags prior to flower opening, to prevent contamination with foreign pollen. Pollen of monoecious or dioecious wind-pollinated plants, such

Plate 1 Flower of hybrid between *Acacia auriculiformis* and *A. mangium* from Ulu Kukut, Sabah, Malaysia, showing small petals, numerous anthers and long tapering style (left). Scanning electron micrograph.

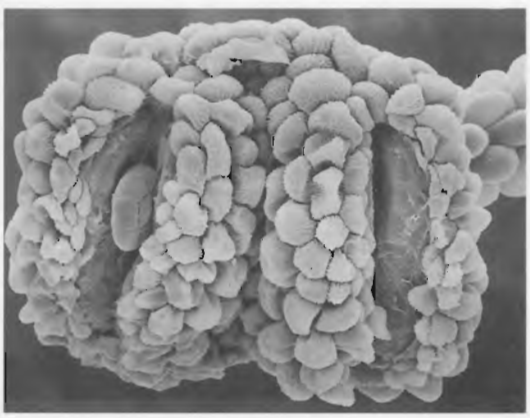

Plate 2 Anther of *Acacia auriculiformis* with polyad in left locule. Scanning electron micrograph.

* Department of Horticulture, Viticulture and Oenology, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, SA 5064, Australia

† Rose Smith died in a hot air balloon accident at Alice Springs in August 1989.

as pine, is the easiest to collect as it is readily dispersed from the anthers of the unisexual flowers, and can be collected directly onto clean trays or white paper. Insect-pollinated species with hermaphrodite flowers present greater problems in pollen collection. Acacias are insect pollinated (Plate 10), and the sticky pollen adheres to the insect body (Plate 11). The pollen does not fall freely from the anther and it must be separated from the other floral parts such as the petals and the pistils. For plants with large blooms, the flowers are collected, dried and then gently rubbed over a coarse mesh of approximately 1–3 mm apertures. This separates the anthers from the petals and pistils. The anthers are then further dried and sieved to remove the pollen. This latter stage alone is sufficient for acacias. The genotype of the pollen for pollination is very important, and correct labelling is essential at all stages of pollen collection. Pollen identification checks can be made using a microscope, as the pollen grain of a particular species has a characteristic appearance.

Plate 3 Curved style and blunt stigma of hybrid between *Acacia auriculiformis* and *A. mangium*. Scanning electron micrograph.

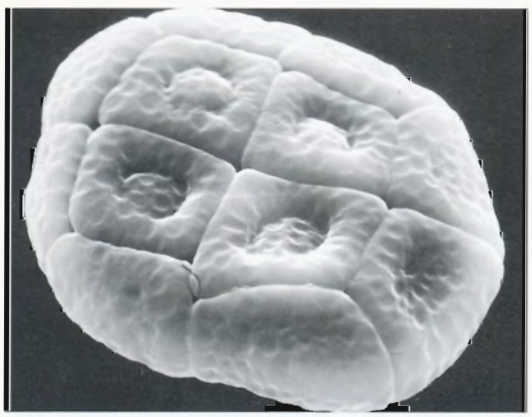

Plate 4 Polyad of *Acacia mangium* consisting of 16 pollen grains. Scanning electron micrograph.

Hybridisation Techniques

The crossing of tree species poses particular problems in that the flowers are generally borne in the canopy of the plant. Scaffolding and pollination platforms are commonly erected around trees selected to be used as the female parent (Plates 12, 13 & 14). The details of the hybridisation method differ from plant to plant depending upon the characteristics of the breeding system, and research is needed to determine the most efficient method. Aspects such as the maximum receptivity period of the flower are particularly important. The first stage of the hybridisation technique is the bagging or isolation of the flower which is to

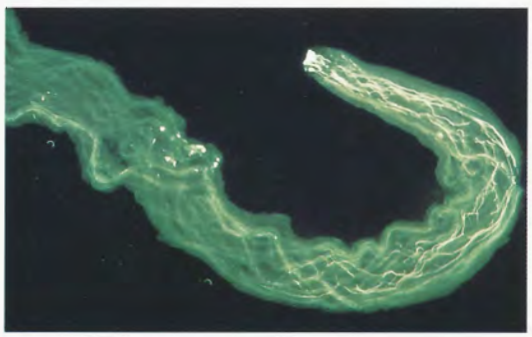

Plate 5 Style of hybrid between *Acacia auriculiformis* and *A. mangium* showing germinated polyad and pollen tubes growing through the style. Fluorescence micrograph.

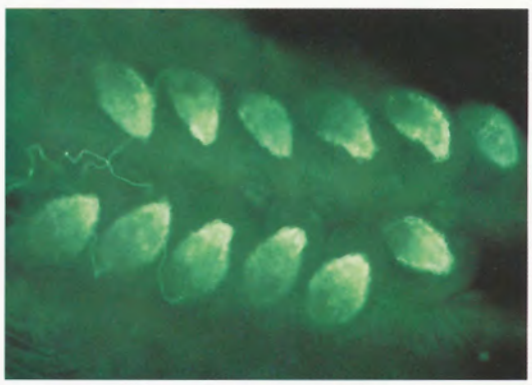

Plate 6 Ovary of *Acacia mangium* showing ovules and pollen tubes. Fluorescence micrograph.

Plate 7 Pod set of *Acacia mangium*.

Plate 8 Mature pods of *Acacia auriculiformis*.

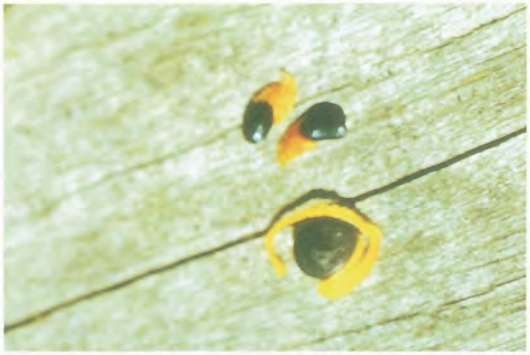

Plate 9 Seed of *Acacia mangium* (top) and *A. auriculiformis* (bottom).

Plate 10 Colletid bee collected from *Acacia mangium* flowers at Tawau, Sabah, Malaysia. Scanning electron micrograph.

receive the pollen. This is done before the flower opens to prevent unwanted pollination by either wind or insect. The bag must be a barrier to pollen but should allow some movement of gases to prevent excessive heat, moisture and carbon dioxide buildup within the bag. Bagging is only important in insect-pollinated species if insects have access to the flowers. This step can be omitted if the breeding plants are kept in an insect-free environment such as a sealed glasshouse. Because of the time taken to bag the flowers there have been attempts to omit this step even in the outdoor crossing of insect-pollinated species. Contamination with foreign pollen in teak breeding is only 1.3% which is an acceptable level in view of the increase in the number of flowers which can be handled per day. Emasculation is an essential step in the crossing of hermaphrodite flowers in order to prevent self-pollination. It must be done prior to anther dehiscence so is generally done in the bud stage. In most species it is convenient to remove the perianth as well as the stamens as this is quicker than prising open the petals and removing the anthers individually. In plants with large flowers specialised emasculation tools made from modified scissors or cutting blades, have been developed which remove all the stamens with one cut. Emasculation is not necessary in species which have strong outbreeding mechanisms such as self-incompatibility or dichogamy. Pollen is generally transferred from the anther to the stigma by hand. In insect-pollinated species a brush is the most popular application tool, although brushing the anther directly against the stigma greatly reduces the pollen preparation time involved. All implements should be sterilised in alcohol when changing from one pollen source to another to prevent contamination. Following pollination the flower is rebagged until either the style is shed or fruit set has occurred. The bag is removed as soon as the possibility of stray pollination is over, so as to avoid problems of buildup of pests and diseases, altered atmosphere within the bag or of rubbing against the branch or flower. The rebagging step can be omitted

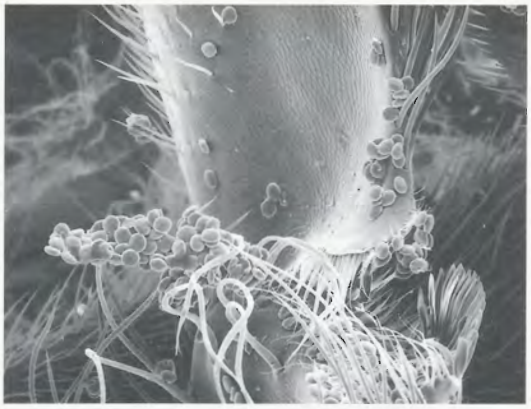

Plate 11 Polyads of *Acacia mangium* sticking to the back legs of a Colletid bee. Scanning electron micrograph.

in some cases where contamination is low, and increased fruit set results from the reduced handling of delicate flowers. When the seed is mature, it is collected and germinated. The success of hybridisation can often be assessed by testing the seedlings using biochemical tests such as isozyme analysis (Plate 15). Even where superior parents are crossed, the progeny may be very variable, and selection for vigour can be commenced at the seedling stage. Variability is particularly likely in the case of interspecific hybridisation (Plates 16 & 17).

Seed set is often low when difficult combinations, such as interspecific or intergeneric crosses are attempted.

Plate 12 Pollination platform attached to a vehicle.

Plate 13 Pollination scaffold.

Plate 14 Detail of pollination scaffold.

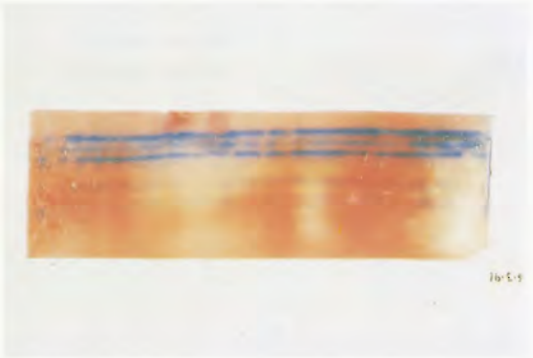

Plate 15 Isozyme electrophoresis gel stained for the enzyme aspartate amino transferase showing hybrid genotype of *Acacia auriculiformis* × *A. mangium* seedling material.

One of the major causes of the low seed recovery is the shedding of immature fruit rather than failure of fertilisation. The embryos in the shed fruit are often viable but incapable of germination due to their immaturity. In this case tissue culture methods can be used to increase the proportion of successful crosses. Embryo culture of immature embryos from shed fruit has been used to salvage progeny of intraspecific crosses in avocado and citrus, and is also used for the culture of embryos from early-ripening cultivars of plum, peach and cherry. Interspecific hybrids have been generated by this method in camellia and walnut.

The techniques of molecular biology also have application in the improvement of tree species, to generate linkage maps, or as biochemical markers similar to isozymes. In the longer term gene transfer may also be a possibility.

Plate 16 Interspecific hybrid seedlings between *Acacia mangium* and *A. auriculiformis*.

Plate 17 Interspecific hybrid seedlings of *Acacia mangium* × *A. auriculiformis* showing variability in seedling growth within a single family.

Hybridisation of Acacias

The technique developed for hybridisation (Plate 18) of *Acacia mangium* (Plates 19 & 20) and *Acacia auriculiformis* (Plates 21 & 22) is as follows:

Step 1 Select spikes as they change from green to yellow (stage c in Plate 23).

Step 2 Label and bag a group of spikes to be used as the female parent (Plate 24). Single spikes should not be bagged as they are too fragile to support a bag. Spikes should not be bagged in the rain, or if they are damp from morning rain, as this will result in rotting of the spike. The window of the bag should face down to prevent buildup of condensation on the window.

Step 3 Label and bag a group of spikes to be used as the male parent.

Step 4 At anthesis (following day) spikes which are not to be used are trimmed from around the experimental spikes to be used as female parents with sharp scissors.

Step 5 Reduce the number of florets on each spike. This is done by gently running the forceps along the side of the spike to reduce the number of florets to approximately 20, and leaving the spike with florets in two dimensions only. This avoids twisting of the spike during manipulations.

Step 6 Any unopened buds are removed by gently rubbing the spike between the fingers.

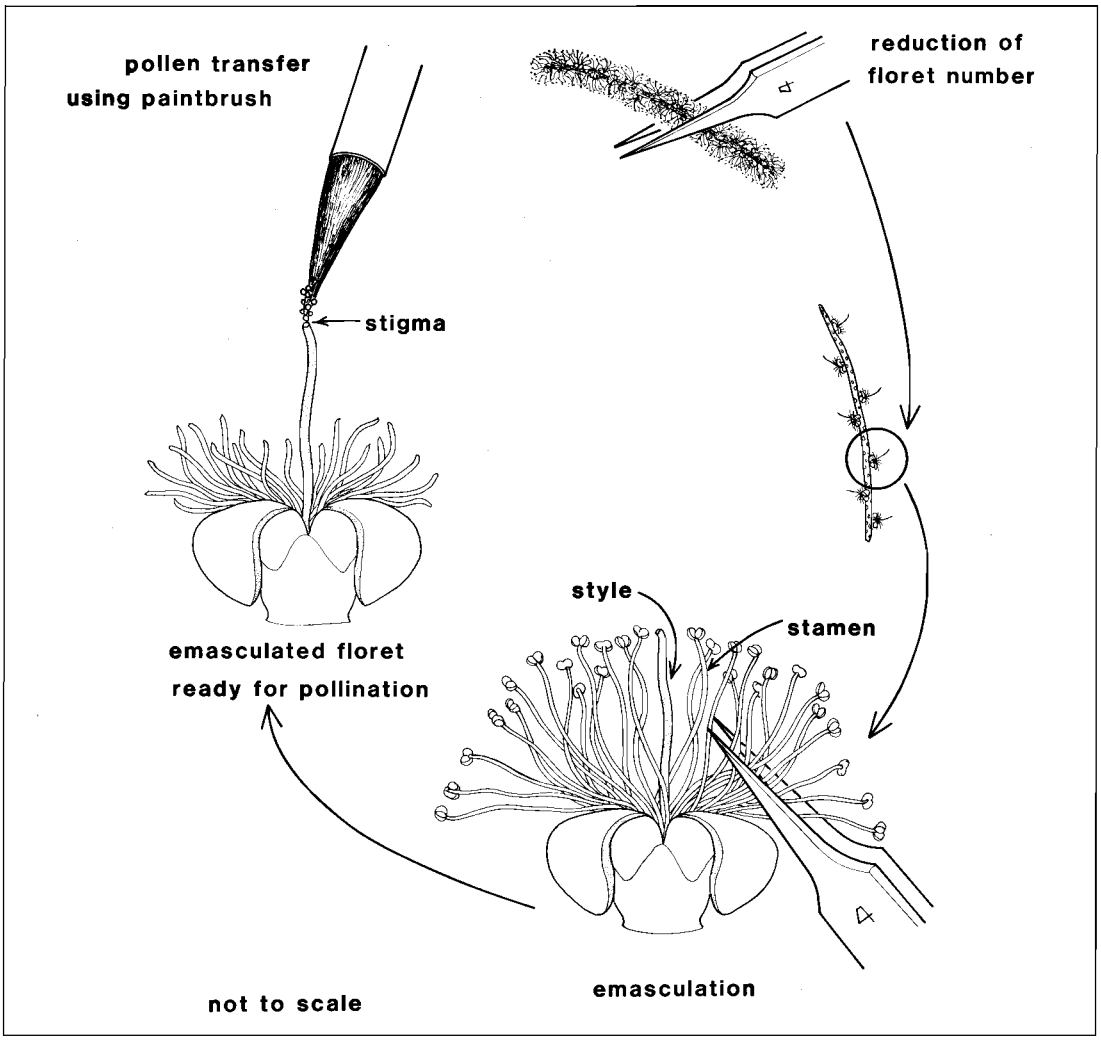

Plate 18 Emasculation and pollen transfer technique for hybridisation of acacias.

Step 7 The remaining florets (between 5 and 30 per spike) are then emasculated by removing the stamens from around the style using fine forceps (Plate 25 & 26).

Step 8 The success of emasculations is measured by checking for contamination with self pollen on each stigma using a 30 × light-scope (Plate 28).

Step 9 At anthesis male spikes are collected and air dried by spreading out on a dry surface in dappled shade for up to four hours until the plant material is dry.

Step 10 Sieve spikes through a 53 micron stainless steel sieve (Plate 27).

Step 11 Collect pollen on a smooth black surface (e.g. black plastic lid from glass jar) on which the yellow pollen can be easily seen (Plate 28).

Step 12 Pollen is picked up with a fine brush with black hairs against which the pollen can be seen.

Step 13 Transfer pollen to a stigma (Plate 29).

Step 14 The presence of a polyad is assessed with the lightscope (Plates 28 & 30).

Step 15 Spikes are rebagged.

Step 16 The branches carrying the experimental spikes are labelled with numbered tags.

Step 17 Remove the bags after three days.

Step 18 Monitor pod development and harvest when mature.

Plate 19 Tree of *Acacia mangium*.

Plate 20 Spikes of *Acacia mangium*.

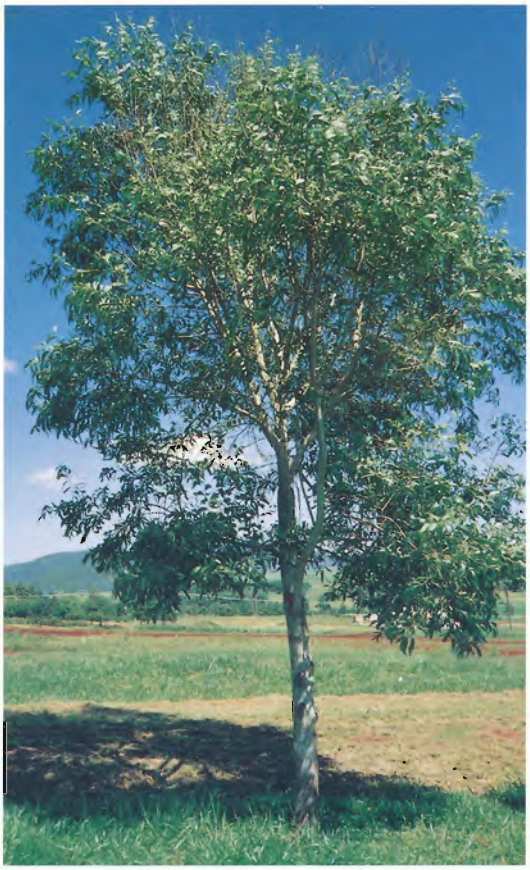

Plate 21 Tree of *Acacia auriculiformis*.

Plate 22 Spike buds of *Acacia auriculiformis*.

The materials required for this are as follows:

Lightscope — 'National' microscope with light. Catalogue number FF–393E 30 × magnification. Matsushita Electric Industrial Co. Ltd., Japan. PO Box 288, Central Osaka, Japan.

Crossing bags — H1010 Standard grade polyester 24 × 16 cm bag with one clear PVC window 6 × 4 cm placed lengthwise. Duraweld Plant Breeding Supplies, Salter Rd., Eastfield Industrial Estate, Scarborough, North Yorkshire, Y011 3UZ, England. Tel: 0723–584091

Forceps — INOX. Number 4. A. Dumont & Fils. Switzerland. Australian supplier: Australian Entomological Supplies, Factory 8, 3 Flora Street, Corner bath Road, Kirrawee, NSW 2232. Tel: (02) 521 8703, Fax: (02) 521 7341.

Sieve — Laboratory test sieve. Stainless steel frame, stainless steel 53 micron aperture mesh. Endecotts Ltd. London England BS410/1986.

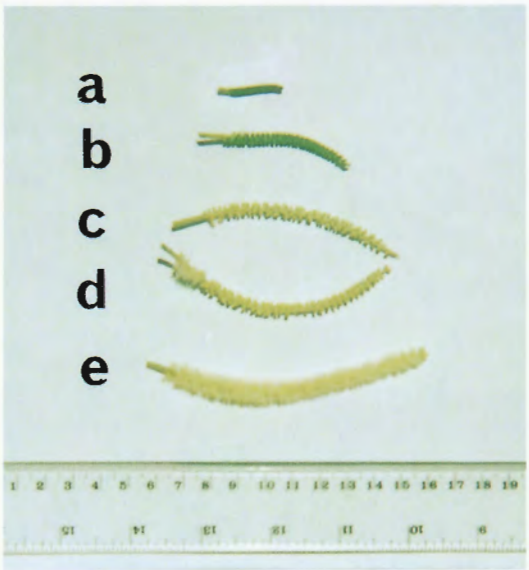

Plate 23 Spikes of *Acacia mangium* at different stages of development: a, immature; b, one week prior to anthesis; c, one day prior to anthesis; d, anthesis of 10% of spike; e, anthesis of whole spike. Spikes are bagged for hybridisation at stage c.

Plate 24 Pollination bags on flowering spikes of *Acacia auriculiformis*.

Plate 25 Emasculating flowers of *Acacia auriculiformis*.

Plate 26 Emasculating flowers of the hybrid between *Acacia mangium* and *A. auriculiformis*.

Brush — a fine paint brush with black hairs against which pollen can be seen

Work surface — any smooth black plastic surface against which the pollen can easily be seen after sieving

Scissors — 5 cm blades

Plastic labels

Further Reading

Carron, L.T. and Aken, Kron (ed.). 1992. Breeding Technologies for Tropical Acacias. ACIAR Proceedings No. 37.

Sedgley, M. and Griffin, A. R. 1989. Sexual Reproduction of Tree Crops. Academic Press, London, 378 p.

Turnbull, J. W. 1987. Australian Acacias in Developing Countries. ACIAR Proceedings No. 16.

Plate 27 Sieving pollen of *Acacia mangium*.

Plate 29 Pollination using a paint brush.

Plate 28 Pollen of *Acacia mangium* collected in a black plastic lid (left hand), and lightscope (right hand).

Plate 30 Polyad on the stigma of *Acacia auriculiformis*. Scanning electron micrograph.

ACIAR Technical Reports

No. 1. ACIAR Grain Storage Research Program: research report 1983-84, 63p., 1985.

No. 2. Pastures in Vanuatu, D. Macfarlane and M. Shelton, 32p., 1986.

No. 3. ACIAR Grain Storage Research Program: research report 1984-85, 85p., 1986.

No. 4. Coconut germplasm in the South Pacific Islands, M.A. Foale, 23p., 1987.

No. 5. South Pacific agriculture: challenges and opportunities for ACIAR and its research partners, G.J. Persley and P. Ferrar, 87p., 1987.

No. 6. ACIAR Grain Storage Reseach Program: research report 1985-86, 96p., 1987.

No. 7. Building on success: agricultural research, technology, and policy for development: report of a symposium held at Canberra, 14 May 1987, J.G. Ryan, 39p., 1987.

No. 8. New technologies for rainfed rice-based farming systems in the Philippines and Sri Lanka: report of a workshop held at Iloilo, Philippines, 20-24 July 1987, 39p., 1988.

No. 9. Gaseous nitrogen loss from urea fertilizers in Asian cropping systems, J.R. Freney, J.R. Simpson, Zhu Zhao-liang and Aziz Bidin, 16p., 1989.

No. 10. Bulk handling of paddy and rice in Malaysia: an economic analysis, G.J. Ryland and K.M. Menz, 32p., 1989.

No. 11. Economic prospects for vanilla in the South Pacific, K.M. Menz and E.M. Fleming, 14p., 1989.

No. 12. Biological control of *Salvinia molesta* in Sri Lanka: an assessment of costs and benefits, J.A. Doeleman, 14p., 1989.

No. 13. Rainfed rice production in the Philippines: a combined agronomic economic study of Antique Province, K.M. Menz, 90p., 1989.

No. 14. Transport and storage of fruit and vegetables in Papua New Guinea, K.J. Scott and G. Atkinson, 22p., 1989.

No. 15. Marketing perspectives on a potential Pacific spice industry, Grant Vinning, 59p., 1990.

No. 16. Mineral nutrition of food legumes in Thailand with particular reference to micronutrients, R.W. Bell et al., 52p., 1990.

No. 17. Rice production in Sri Lanka, K.M. Menz (ed.) 51p., 1990.

No. 18. Post-flask management of tissue-cultured bananas, Jeff Daniells and Mike Smith, 8p., 1991.

No. 19 The utilisation of remote sensing in the South Pacific, D. van R. Claasen, 59p., 1992.