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Breeding Technologies for Tropical Acacias

Proceedings of an international workshop held in Tawau, Sabah, Malaysia, 1–4 July 1991

Editors: **L.T. Carron and K.M. Aken**

Organised by staff of:

Sabah Plantation Forestry Committee (SPFC)
Innoprise Corporation Sdn Bhd (ICSB)
Sabah Forest Industries (SFI)
Sabah Softwoods Sdn Bhd (SSSB)
Forest Research Centre (FRC), Forestry Department Sabah
Sabah Forestry Development Authority (SAFODA)

Forest Research Institute of Malaysia (FRIM)

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Contents

Foreword

Fast-growing acacias are being used to reforest degraded grasslands and cutover rainforest in the humid tropics. *Acacia mangium* has been planted extensively in Malaysia and Indonesia in recent years to provide timber or raw material for the pulp and paper industry. *Acacia auriculiformis* has been an important exotic in many tropical countries for more than half a century. It is widely planted for fuelwood, erosion control, shade, shelter and amenity, but with selection and breeding it has great potential to provide timber and other industrial wood products.

In 1989 the Government of Malaysia signed an agreement with the Australian Centre for International Agricultural Research (ACIAR) to undertake a collaborative research project entitled 'Hybridisation and Vegetative Propagation of Australian Tropical Acacias'. This project brought together scientists from CSIRO Division of Forestry, University of Adelaide, Forest Research Institute of Malaysia and the various government agencies and enterprises which make up the Sabah Plantation Forestry Committee. The project aimed to develop a thorough knowledge of the floral biology of *A. mangium* and *A. auriculiformis* as the basis for the manipulated production of hybrids between selected trees, and to evaluate the potential for hybrid seed production in orchards. It further sought to develop mass vegetative propagation techniques using both cuttings and micro-propagation so that selected hybrids can be multiplied rapidly.

The workshop was designed to provide a mechanism for the exchange of research methods and results among the project scientists and between the scientists and their clients. It was also a forum in which to discuss and formulate future research directions.

ACIAR sponsored the workshop as part of its Forestry Program. Further support was provided by CSIRO's Australian Tree Seed Centre through its AIDAB project 'Seeds of Australian Trees'. ACIAR wishes to thank Mr Cyril Pinso (Innoprise Corporation Sdn Bhd) and his committee for organisation of the workshop and Sabah Softwoods Sdn Bhd for hosting the workshop field day and the post-workshop training course.

The 52 participants and observers at the workshop came from Australia, People's Republic of China, France, Indonesia, Laos, Malaysia, Sri Lanka, Thailand, United Kingdom and Vietnam. ACIAR thanks those who presented papers and contributed to the discussion. The project results represent a very significant advance in developing breeding technologies for tropical acacias.

The contributions of Peter Lynch for editorial direction and of Maureen Kenning for coordination of the preparation of these proceedings are greatly appreciated.

G.H.L. Rothschild
Director
ACIAR

Future Directions

Future Directions for Research, Development and Training

D.G. Nikles*

BASIC technologies required to implement a program of breeding, propagating and testing interspecific *Acacia* hybrids have been developed in ACIAR project 8630, though it is recognised that further monitoring of some experiments is necessary and some new work would be highly desirable.

The overall objectives of follow-up research required to underpin/support future operational breeding and which could be achieved within the next four years are:

- Establishment of field trials which would adequately quantify the performance characteristics of a few, putatively superior interspecific hybrids (in addition to *A. mangium* × *A. auriculiformis* and its crosses to other species such as *A. aulacocarpa*) and controls, and estimation of population parameters needed to model and evaluate alternative breeding and propagation strategies.
- Development of appropriate, interim breeding and propagation strategies mainly through consultative services, and the training of scientists to become qualified forest tree-breeding strategists.

An appropriate new program of research and development for a breeding program of tropical acacias should comprise five components as follows:

Component 1

Complete certain studies on reproductive biology and vegetative propagation started under ACIAR project 8630, and undertake some new work.

Reproductive biology

For future research work with hybrids of tropical acacias, and for future operational breeding and propagation at an intensive level, it will be essential to have a reliable means for controlled crossing. This in turn will involve improved pollen handling procedures of which the most pressing aspect is determination of workable pollen storage and testing techniques. Component (2), the largest experimental activity of the recommended new research Project, involves research crossing which would be greatly facilitated by availability of viable, stored pollen at various times.

For less intensive breeding it may not be essential to emasculate and thus guarantee 100% pure hybrid or intraspecific, out-crossed seed. It has been found that a significant amount of selfing is feasible if flowers are not emasculated and protected from sources of self pollen. Experience with *A. mearnsii* at the Wattle Research Institute (now the Institute of Commercial Forestry Research) in South Africa was that a small proportion of selfs usually occurred when there was no emasculation. Although selfs could be detected as various mutants in the progeny of some genotypes carrying marker genes, it was not so for all selected genotypes of *A. mearnsii*. Thus it would be very useful to check the hypothesis that, for less intensive operational breeding and propagation purposes, controlled crossing without emasculation is acceptable. A positive finding in such a study could open up new possibilities such as producing both pure breed and hybrid progeny in stands which were intimate mixtures of retained superior seedling or cloned trees — an approach which would be useful. Thus it is suggested that new work investigating simple technology for hybrid production be undertaken.

Work on the established biclonal orchards, by which a useful proportion of hybrids might be produced, should continue. For two successive years all mature pod crops in each orchard should be sampled, and seedlings assayed for hybridity (by means of the isozyme technology and morphological criteria developed under the Project). Accumulated results should then be analysed, interpreted and reported.

New work on mass production of hybrid seed should be undertaken such as use of more than one pollinator clone in seed orchards and possibly on the use of gametocides to prevent effective pollen dispersal by the seed parent clones.

Further development of isozyme or other technology seems necessary to check on the hybridity of individuals in crosses produced without emasculation and of progeny

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from the biclonal orchards, and for definition of hybrid genotypes in the set of interspecific crosses of component (2).

Vegetative propagation

It is noted that the technology for rooting juvenile cuttings, developed in Project 8630, has already been employed in practice in Indonesia. The P.T. Indah Kiat Company in Sumatra established some 800 ha of rooted cuttings in 1991.

The field trials of rooted cuttings established under Project 8630 need to be monitored and assessed, and results analysed and interpreted. Since the trials were planted as recently as 1991 such monitoring should continue until the end of 1994.

Several other aspects of the vegetative propagation studies should be continued, including field performance tests of rooted cuttings from selection-age trees, rooting responses through more generations, assessment of G × E interaction of rooted cuttings, and accumulation of information needed to make decisions on breeding and propagation options. Studies of the latter aspect would be possible using material proposed for production and testing as the main thrust of a PhD research project. See component (4).

The work on comparing seedlings and rooted cuttings should be repeated, this time using fully comparable genetic materials.

The work on micropropagation need not be carried further in an ACIAR project, because there are strong indications that macropropagation techniques are adequate (Haines and Griffin, these proceedings); micropropagation work will continue at FRIM; and it is to be instituted at Tawau in the second half of 1991 by Innoprise Corporation and the Centre Technique Forestier Tropical (CTFT).

All of the above studies should be applied to hybrid acacias.

Component 2

Groups in Sabah should plan and implement a cooperative study designed to produce, test and quantify the potential superiority of interspecific hybrids among selected Australian tropical acacias, and to provide estimates of population parameters. This component should incorporate field testing of several hybrids and controls at appropriate places in Sabah, in some states in Peninsular Malaysia, and in Indonesia and Papua New Guinea, where *Acacia* species are being planted. Studies of self-incompatibility should also be undertaken. Appropriate mating and field designs will be essential.

Although the *A. mangium* × *A. auriculiformis* F_1 hybrid shows promise, it is the only one of several possible interspecific combinations among tropical acacias which has been studied for performance. Using information provided by Sim Boon Liang of Sabah Forest Industries SB and others, which rated the species and the *A. mangium* × *A. auriculiformis* hybrid for important traits on a 1–10 scale, Nikles and Griffin (these proceedings) showed that some pairs of the Australian tropical acacias exhibit complementary desirable characteristics which might be combined in hybrids, resulting in superior overall performance.

Pairs of taxa with particularly high likelihood of hybrid superiority include *A. mangium* and *A. aulacocarpa* and the *A. mangium* × *A. auriculiformis* F_1 hybrid crossed to *A. aulacocarpa*. Other species with some highly desirable characteristics include *A. crassicarpa*, *A. leptocarpa* and *A. polystachya*. The question of which interspecific combination or combinations are 'best' is further complicated by the genetic variation among and within provenances, and the likelihood of important genotype-by-environment interaction across the wide range of conditions in southeast Asia where tropical acacias and hybrids are being grown. Therefore it is contended that, in parallel with any development to operational use of the *A. mangium* × *A. auriculiformis* hybrid, it should be compared in rigorous field tests with other interspecific combinations.

Groups in Sabah have demonstrated a capacity to collaborate in research and development on *Acacia* biology, breeding and propagation within ACIAR project 8630, as well as on other aspects of plantation development. Several groups have committed themselves to genetic improvement programs, and at least two are aware of the potential value of national cooperation in genetic improvement of *Acacia* (Pinso, these proceedings; Sim, these proceedings). These groups have between them sufficient genetic resources of *Acacia* and the human resources required to make the interspecific crosses and controls needed for a suitable study.

It is anticipated that a limited number of species (each represented by several clones of each of two provenances) plus the *A. mangium* × *A. auriculiformis* F_1 hybrid would be selected for the study, and an appropriate systemic mating design chosen for use. Ideally the very considerable crossing program required would be carried out via cooperation among four or five agencies in Sabah. This would render the task of each agency quite feasible because any one agency would be required to make only a relatively small number of crosses using clones in its own clone banks. Accomplishing the required crosses might take two years, so a total of three years would have to be allowed for the first stage of planning and implementing the crossing and accumulating all the seed. A second phase would involve development of standard nursery and field test designs, selection of cooperators inside and outside Sabah, distribution of seed, and establishment and maintenance of field trials.

Such a series of well-planned field trials would enable estimates of costs and of various population parameters to be made, estimates which are required inter alia for developing appropriate breeding and propagation strategies. The proposed study would also reveal any breeding barriers between species. A very similar study has been undertaken at the Australia–China Eucalypt Afforestation Project at Dongmen, in southern China, involving five eucalypt species and an F_1 hybrid between two of them. It has produced and is testing six interspecific F_1 and two back-cross hybrids.

Component 3

Groups in Sabah, Peninsular Malaysia and Indonesia (at least) desiring the development and documentation of appropriate breeding and propagation strategies for short- and long-term genetic improvement of *Acacia* species and hybrids should secure the services of appropriate consultants.

There is general agreement in Malaysia that much of the benefit of ACIAR Project 8630 will materialise only if an operational hybrid breeding program is developed. If organisations in Malaysia take the initiative to start such work, with assistance from ACIAR, there will be mutual benefit. Some Australian workers have research and/or operational programs for the co-improvement of pairs of species for superior hybrid development, and the experience of such workers would be of great benefit to Malaysia. At the same time, Australian workers would gain additional experience in working with colleagues in Malaysia on different species, namely Australian tropical *Acacia* species, the reproductive biology and growth of which allow results to be obtained quickly.

Component 4

Groups in Sabah, Peninsular Malaysia and Indonesia (at least) need to select and train one or more persons to become forest tree breeding strategists, preferably at PhD level.

In line with experience elsewhere of the economic gains to be obtained through tree improvement, there is a need for each organisation in Malaysia and Indonesia, which has large planting programs with *Acacia* species, and may use hybrids in the future, to develop and implement appropriate breeding and propagation strategies. Such strategies could be easily modified if a cooperative tree improvement program was established. As inferred under Component (3) above, there are no practising expert forest tree breeding strategists in Malaysia and Indonesia at present. There are good reasons for training in Australia suitable candidates to become forest tree breeding strategists to work specifically with *Acacia* in Malaysia and Indonesia. These reasons include:

- There is already a considerable history of Malaysian-Indonesian-Australian collaboration in development of *Acacia* genetic resources.
- There has been or is currently a considerable amount of work by CSIRO Division of Forestry, Canberra, and the Queensland Forest Service on seed collection, variation, breeding systems and establishment of seed orchards of Australian tropical acacias.
- Good opportunities exist at some Australian universities and CSIRO, either singly or in collaboration, for work on the genetic parameters of *Acacia* populations (pure and hybrid).
- In at least one such university (University of Queensland) there are available advanced courses in Genetics and Quantitative Genetics which have been undertaken by several forestry graduates in recent years, and supervision of internal and external students to the PhD level in forest tree breeding can be arranged in association with the Queensland Forest Service. It would be appropriate for Malaysian and Indonesian students to work on problems such as evaluation of alternative breeding strategies for genetic development of pure and hybrid breeds of tropical acacias. Breeding strategists trained in this way would, of course, be capable of developing and reviewing strategies for genetic improvement of other tree species as well.

Component 5

Breeding strategists trained as outlined above would, of course, be capable of applying their knowledge and experience to the development or review of strategies for genetic improvement of other tree species as well as training at professional and technical levels in relevant areas of research and development planning, and in technologies required in a tree improvement program.

Benefits from Project 8630 would be extended if training workshops were conducted in Indonesia, and relevant places elsewhere, aiming to develop expertise locally in all aspects of the technologies related to effective control of pollination and vegetative propagation. Other important areas for training local workers are data gathering, storage, analysis, interpretation, reporting and use; and design of field and other trials. Such training could be incorporated in an extended or new ACIAR project.

Maximum benefit from the availability of seed and cuttings of superior tropical acacia hybrids will only be realised if there is optimal follow-on integration of appropriate technologies at the nursery, site preparation, establishment, management (including protection) and utilisation phases of forest development and use. As necessary, therefore, there should be parallel research, development and training in these fields.

Introduction

An Outline of the Organisational Content of ACIAR Project 8630

Wan Razali Wan Mohd,* A.R. Griffin† and R. Wickneswari*

THE potential of *Acacia mangium* Willd. as a plantation species was first demonstrated in Sabah, Malaysia, and by 1985 about 20 000 ha had been planted (Udarbe and Hepburn 1986). In one of the early plantings at Ulu Kukut, the species grew in close promixity to *A. auriculiformis* A. Cunn. ex Benth. planted as firebreaks and windbreaks. Hybrid individuals were subsequently observed in plantations derived from the Ulu Kukut *A. mangium* seed. These hybrids had a number of desirable intermediate features (Rufelds and Lapongan 1986). They tended to grow vigorously and have better form than *A. auriculiformis*, with lighter branching than *A. mangium*.

An opportunity for systematic hybrid breeding was thus identified. This idea was promulgated by breeders from Sabah at an ACIAR Acacia Workshop at Gympie in 1986. A decision was taken at the meeting to develop a proposal which addressed the problem of breeding improved hybrids between *A. mangium* and *A. auriculiformis*. Agreement for the current three-year project on Hybridisation and Vegetative Propagation of Australian Tropical Acacias was reached between ACIAR and the Malaysian Government on 1 December 1988.

Organisational Structure

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Waite Agricultural Research Institute (WARI) have coordinated the project on behalf of ACIAR. The Forest Research Institute Malaysia (FRIM), in collaboration with the Sabah Plantation Forestry Committee (SPFC), has accomplished this role on behalf of the Government of Malaysia. The Australian project coordinators are Dr A.R. Griffin of CSIRO and Dr M. Sedgley of WARI; the Malaysian coordinators are Dr. Wan Razali Wan Mohd of FRIM and Mr Cyril Pinso of Innoprise Corporation Sendirian Berhad (ICSB) (this role was initially assumed by the late Mr J. Hepburn of SAFODA) representing the interests of SPFC. Dr A.R. Griffin was the overall coordinator of the project until he left CSIRO employment in January 1991. Mr. Kron Aken of the CSIRO Australian Tree Seed Centre then assumed this responsibility.

Once the work program was agreed the Malaysian scientists in both Sabah and FRIM worked in direct communication with their Australian counterparts, with visits in either direction as necessary. In Sabah the SPFC provided an effective forum for review and planning of research activities carried out by member organisations, viz. Sabah Softwood Sendirian Berhad (SSSB), Sabah Development Authority (SAFODA) and Sabah Research Centre (SFRC).

An important principle established from the outset was that, since this was a collaborative project of mutual value to both the Malaysian and Australian scientists, Malaysian organisations would in general contribute staff time and in-country operating expenses. This maximised the project budget available for travel between the countries for training, consultation and review meetings. The major exceptions were the support of skilled assistants for Dr Sedgley's field and laboratory program and Dr Wickneswari's isozyme laboratory at FRIM. Of a total approved budget (including variations) of $A289 027 (M$606 957) for this project, about $A74 135 (M$155 684) was used for the Malaysia program and the remaining $A214 892 (M$451 273) was used for the Australia program.

Research Objectives and Activities

The breeding of improved interspecific hybrids generally requires mastery of controlled pollination techniques for producing progeny for evaluation trials, together with an ability to mass propagate the clones which are subsequently selected. Development of these methodologies was identified as the broad aim of the project — with a view to technically equipping breeders in Malaysia to commence operational hybrid breeding programs. Since such knowledge is also generally lacking for *A. mangium* and *A. auriculiformis*, the project also

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contributes to improvement strategy options for these species. The specific objectives of this project were:

1. To develop a reliable manipulated hybridisation technique for tropical acacias and to evaluate potential for open-pollinated hybridisation in seed orchards.
2. To develop methods for mass vegetative propagation of tropical acacias and their hybrids as a means of rapidly capturing genetic gains from breeding.

Reproductive Biology

To successfully manage seed production it is necessary to have a thorough understanding of the floral biology of each parent species. Thus the program also called for field observations on phenology and of the insect species which were effecting pollination in Malaysia and Australia. Detailed studies of the morphology, anatomy, temporal development and incompatibility systems, using electron and light microscopy techniques, were carried out to provide a basis for developing manipulated hybridisation procedures.

Bulk quantities of hybrid seed might be produced by open pollination if clones of each species flower at the same time and are at least moderately self-incompatible. A series of biclonal hybridising orchards was planted by SSSB to evaluate this option. Since operational use would depend upon the nurseryman's ability to recognise hybrid seedlings in contrast with those from the parental species, detailed seedling morphology studies were carried out at SFRC and subsequently further developed following independent evaluation in six of the collaborators' nurseries. Isozyme analysis provided a valuable research tool for determining hybridisation and outcrossing rates under open pollination and for verifying the hybrid origin of seed produced by manipulation. This capability was developed at FRIM.

Vegetative Propagation

Macropropagation techniques were already being tried at SSSB at the outset of the project and a major effort was put into developing these to an operational level. The plan called for particular investigation of the multiplication rate from young seedlings and of age effects on rooting ability of shoots from coppiced trees. The latter is important because the clonal propagation of trees which are shown to be superior in evaluation trials offers the most rapid means of capturing genetic gains from breeding.

Table 1. Contribution of Malaysian and Australian organisations to components of the research program for ACIAR Project 8630.

Activity	Organisation								
	Malaysia						Australia		
	FRIM	SFRC	SSSB	SAFODA	SFI	LFC	CSIRO	WARI	QFS
Reproductive biology									
Floral biology	•							•	
Manipulated hybridisation	•		•						
Phenology	•	•	•	•				•	
Pollinators	•	•	•					•	
Isozymes	•						•	•	
Hybridising orchards	•	•	•				•		
Seedling morphology study	•	•	•	•	•	•			
Vegetative propagation									
Cuttings			•						•
Marcotting		•	•	•					
Micropropagation	•								

Key: FRIM — Forest Research Institute Malaysia, Kepong, 52109 Kuala Lumpur, Malaysia; SFRC — Sabah Forest Research Centre, Sepilok, Sabah, Malaysia; SSSB — Sabah Softwoods Sdn Bhn; PO Box 137, 91007 Tawau, Sabah, Malaysia; SAFODA — Sabah Forestry Development Authority, Bangunan Expo, Lot 12, Jalan Tun Fuad, Teluk Likas, Beg Berkunci 122, 88999 Kota Kinabalu, Sabah Malaysia; SFI — Sabah Forest Industries, WDT 31, 89859 Sipitang, Sabah, Malaysia; LFC — Luasang Forestry Centre, Tawau, PO Box 795, 91008 Tawau; CSIRO — CSIRO Division of Forestry, PO Box 4008, Canberra, ACT 2600, Australia; WARI — Waite Agriculture Research Institute, University of Adelaide, Glen Osmond, S.A. 5064, Australia; QFS — Queensland Forest Service, Forestry Research Centre, MS 483, Gympie, Qld 4570, Australia.

Micropropagation has received wide attention as a means of bulking up numbers of particular genotypes more rapidly than is possible with cuttings. FRIM has mounted a vigorous program of micropropagation research for the hybrids and part of this work designed to field-test micropropagules in comparison with seedlings and cuttings has been incorporated in the project. As an outcome we will be in a position to recommend appropriate roles for each type of propagation technology in future breeding programs.

The interrelationship of the components of the project are shown in Table 1.

Conclusions

Progress has been reviewed at annual meetings and the present Workshop represents an opportunity for all participants, and other workers from the region, to share information and discuss implications for future work.

The project does not conclude until December 1991 and there are a number of studies still in progress. We are however happy to report that the principal objectives have substantially been achieved.

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The Potential Use of *Acacia mangium* × *Acacia auriculiformis* Hybrid in Sabah

Cyril Pinso* and Robert Nasi†

Historical Background of the Species in Sabah

Acacia mangium

Acacia mangium was first introduced to Sabah in 1967 from a single tree collection from Mission Beach (Queensland). The seedlings (about 570 plants) from these seeds were raised at two sites — Sibuga (200 plants) and Ulu Kukut (370 plants).

At Ulu Kukut, most of the trees (about 300) were planted in December 1967 as a firebreak (known as Jalan Madu); the remaining were planted around the forestry quarters and some were probably sent to the east coast. Two plots were established in November 1967 with the seedlings raised in Sibuga — 100 trees in plot 4B at Gum-Gum and 70 trees in plot 5T at Sibuga.

Up to 1981, all the plantations established in Sabah originated from this unique progeny from Queensland. After a few generations, the need for a wider genetic base arose strongly and new material was brought into Sabah from Queensland, Papua New Guinea and Indonesia. At the same time, seed production areas were developed, especially in Sabah Softwood, to ensure an adequate supply for existing and future planting programs.

The list of the available materials in Sabah is given in Table 1.

Acacia auriculiformis

Little is known about the first introduction of *A. auriculiformis* in Sabah. The species has been cultivated as an ornamental tree for a long time in Sabah, but the first planting trials were probably established around 1953. The original seed sources are unknown but it is believed that most of the plantings before 1965 originated from seeds collected from a few old trees in Labuan. It is therefore possible that the *A. auriculiformis* planted as a firebreak near the *A. mangium* at Ulu Kukut came from the same source and may possess a narrow genetic base.

After 1965, new materials were brought into Sabah from Australia (Queensland, Northern Territory), Papua New Guinea and Thailand. They were established in different parts of Sabah as planting trials.

The list of the available materials in Sabah is given in Table 2.

A. mangium × *A. auriculiformis* hybrid

The first occurrence of a possible hybrid between *A. mangium* and *A. auriculiformis*, hereafter referred to as 'the hybrid', was recognised in 1972 by Messrs Hepburn and Shim amongst the roadside planting in Sook. Later, Tham (1976) stated that the two species hybridise and that the hybrid was confirmed in July 1978 by Pedley (Queensland Herbarium) after looking at a herbarium specimen (SAN 81053) sent to him in January 1977.

The first reaction was to attempt to remove all the *A. auriculiformis* in the vicinity of *A. mangium* from which seed was collected, and to attempt to remove the hybrids from existing planting trials.

The hybrid has since been invariably recorded in areas planted with seed which was collected from Ulu Kukut, where the original natural hybridisation occurred. The occurrence of the hybrid is low but significant (3–4 trees per hectare according to Rufelds, 1987; 1 hybrid per 500 *A. mangium* according to Wong, pers. comm.) and hybrid seedlings are often found in close proximity to adult hybrid trees (Rufelds 1987).

Characteristics of the Hybrid vs the Pure Species

Interspecific hybrids have two principal advantages when compared with the pure species:

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Table 1. *Acacia mangium* material available in Sabah.

Country	Provenance	Location
Indonesia	Cililin	Madahan
Indonesia	Piru	Kolapis A, Telupid, SFI, Bangkol
Indonesia	Sidei	Kolapis A, Telupid, SFI
Papua New Guinea	Balamuk	SSSB, Silam, Mandahan
Papua New Guinea	Bandaber	Luasong
Papua New Guinea	Bensbach-Balamuk Rd	Luasong
Papua New Guinea	Bimadebun	Luasong
Papua New Guinea	Boite	SFI
Papua New Guinea	Derideri	Luasong
Papua New Guinea	Dimisisi	Luasong
Papua New Guinea	Gubam Boite	Luasong
Papua New Guinea	Iokwa	Kolapis A, SSSB, Sook,Telupid, Silam, Madahan
Papua New Guinea	Mai Kussa River	Luasong
Papua New Guinea	Mibini village	Luasong
Papua New Guinea	Oriomo River	Kolapis A, SSSB, Sook, Telupid, Silam, SFI, Bangkol, Kota Beluol, Belud Mandahan, Luasong
Papua New Guinea	Pongaki	Luasong
Papua New Guinea	S. of Keru	Luasong
Papua New Guinea	Toko	SSSB, Silam, Mandahan
Papua New Guinea	Wemenever	SFI
Queensland	Abergowrie	SSSB, Sook Silam, Bangkol
Queensland	Ayton	Luasong
Queensland	Broken Pole Creek	Kolapis A, SSSB, Sook, Telupid, SFI, Bangkol
Queensland	Cardwell	Kolapis B, SSSB
Queensland	Cassowary Creek	Kolapis B, SSSB, Telupid, SFI, Mandahan
Queensland	Claudie River	Kolapis A, SSSB, Sook, Telupid, Silam, SFI, Kota Belud, Mandahan
Queensland	Cowley Beach	Kolapis A, Telupid, SFI
Queensland	Daintree	Kolapis B, SSSB, SFI
Queensland	Ellerbeck Rd, Cardwell	Mandahan, Bangkol, SSSB, Sook
Queensland	Euluma Creek	SSSB
Queensland	Ingham	Luasong
Queensland	Jullaten	Kolapis B, SSSB
Queensland	Mission Beach	Kolapis B, SSSB
Queensland	Mossman	Kolapis B, SSSB, Sook, Bangkol
Queensland	Mourilyan Bay	Kolapis B, SSSB, Bangkol
Queensland	N.W. of Lockhart River	Luasong
Queensland	Olive River	Kolapis A, Sook, Telupid, Kota Belud
Queensland	Rex Range	Kolapis B, SFI
Queensland	Shelburne Bay	Luasong
Queensland	S.W. of Cairns	Luasong
Queensland	Tully Mission	Kolapis B, SSSB, Silam, SFI, Bangkol
Queensland	Wablis Pyramid	Kolapis B, SSSB, Sook, Silam, SFI, Mandahan
Queensland	Whyanbell Creek	SSSB
Sabah	Gum Gum	Silam, SFI
Sabah	Jin Lee	Kolapis A, Sook, Telupid, Bangkol, Kota Belud
Sabah	Jin Madu	Kolapis A, SSSB, Sook,Telupid, Silam, Bangkol, Kota Belud, Mandahan
Sabah	Silam seedstand 1	SFI, Luasong
Sabah	Sook	Kolapis A, Telupid, SFI, Bangkol, Kota Belud, Mandahan
Sabah	SSSB seedstand (55/77)	Kolapis A, SSSB, Sook, Silam, SFI, Kota Belud

- through hybridisation, the breeder can create different gene combinations that may not occur in nature;
- hybrids sometimes exhibit heterosis (or hybrid vigour), in which the hybrid is superior to both parents.

The first advantage exists always, whereas the second is generally rare or not dramatic in forest tree species. The purpose of this second part of the paper will therefore be to review the existence of a possible heterosis for the *A. mangium* × *A. auriculiformis* hybrid.

Unfortunately there are no available data, coming from a well established experiment, by which to compare the hybrid and the pure species. The only quantitative study to date was carried out in 1987 by C. Rufelds at one site (Hobut A, *A. mangium* SAFODA's plantation) in the north of Sabah. There are, however, several records and unpublished observations on the performance of the hybrid in *A. mangium* plantations (Sim Boon Liang, Wong Chin Yong, Khamis Selamat, pers. comm.).

Table 2. *Acacia auriculiformis* material available in Sabah.

Country	Provenance	Location in Sabah
Guam	Yigo	SFI
Northern Territory	Croll Creek	Silam, SFI
Northern Territory	Darwin	Kilapis A, SFI
Papua New Guinea	Balamuk	SFI, Luasong
Papus New Guinea	Bandaber	Luasong
Papua New Guinea	Bula	SFI
Papua New Guinea	Iokwa	SFI
Papua New Guinea	Mai Kussa River	Luasong
Papua New Guinea	Mibini swamp	Luasong
Papua New Guinea	Morehead District	Luasong
Papua New Guinea	Morehead R. Rouku	Luasong
Queensland	Archer River	SSSB, SFI, Luasong
Queensland	Bensbach-Balamuk	SSSB, SFI
Queensland	Morehead River	SSSB, Silam, SFI
Queensland	Rifle Creek	Keningau
Queensland	S. Coen, Cape York	SSSB, Silam, SFI
Sabah	Jin Lee	Keningua
Sabah	Karamunting	Silam, SFI
Thailand	Khao Tha Chud	SFI, Bangkol
Thailand	Muak Lek	SSSB, Silam, SFI
Thailand	Rayong	SFI, Bangkol
Thailand	Saithong Plantation	SFI, Bangkol
Thailand	Waysapung Plantation	SFI, Bangkol

Growth

The first to note a possible heterosis in term of growth was probably Tham in 1976, who stated: 'The hybrid tree tends to be taller than both parents, but retains the poor form of *A. auriculiformis*'. According to Wong (pers. comm.), the F_1 hybrid outperforms *A. mangium* in most of the Sabah Softwood planting sites. In Ulu Kukut, the heterosis is obvious and the natural hybrid is very much taller and bigger than the pure species. However, Rufelds does not find any significant growth differences between the F_1 hybrid and *A. mangium* in her study, which seems to indicate a possible site × heterosis interaction. Moreover, Sim Boon Liang (pers. comm.) claims that the F_2 hybrid shows an important hybrid breakdown and therefore is not generally suitable even though some outstanding individuals are produced. A small plot of hybrid in Muak Lek (ASEAN-Canada Forest Tree Seed Centre, Thailand) shows a better growth than an adjacent *A. auriculiformis* plot.

Despite a certain lack of quantitative data, it seems possible to draw the following conclusions:

- the heterosis is possible but not obligatory and most probably influenced both by the genetic structure of the parent populations and the site;
- the growth performances of the natural F_1 hybrid are better than those of Sabah provenances of *A. mangium*, but not as good as those of exotic provenances such as Oriomo River or Claudie River;
- the growth performances of progenies from F_1 (F_2, F_3, etc.) are very uneven with an average below that of *A. mangium* but with some outstanding individuals.

Form

Generally *A. mangium* possesses a fairly good form with a well defined, relatively straight bole and a good crown:clear bole ratio. It also has some less favourable traits, such as large branches and fluted stems. On the other hand, *A. auriculiformis* is well known for its very poor form and high occurrence of low forking. However, some of the provenances exhibit a very good form (especially some North Queensland provenances) and present generally a light branching habit combined with a good stem circularity.

It is, therefore, obvious that a hybrid combining the best traits of the pure species i.e. straight bole, good crown/clear bole ratio, light branching, good stem circularity, etc. would be of very good value for any planting program.

As for growth traits, the only published study on hybrid qualitative traits is the one carried out by Rufelds in 1987. She concluded that the hybrid traits are intermediate, between the two parent populations, without true heterosis. The hybrid outperforms *A. mangium* in terms of stem circularity, branch diameter and self-pruning ability, whereas *A.mangium* presents better stem straightness, crown shape and branching angle.

Given these reasonably good results and keeping in mind that this study was carried out on an unselected F_1 hybrid from natural crosses between two mediocre and genetically poor parent populations, the production of outstanding populations of hybrid trees seems possible by means of controlled crosses between carefully selected populations. Such a production of hybrid populations possessing suitable form traits of both parent populations will probably be easier than the production of hybrids with outstanding growth because of the strong genetic control on the form traits. Of course, this is directly subordinate to the development of practical controlled pollination techniques.

Wood properties

The wood properties of *A. mangium* and *A. auriculiformis* have been studied and presented in several technical

papers. In brief, the two species are very suitable for chips, pulp and paper, plywood and medium density fibreboard.

Physical and mechanical properties

A. auriculiformis from India has a high specific gravity (0.6–0.7) and better physical and mechanical properties than teak wood (Kumar et al. 1987). *A. mangium* from Ulu Kukut has a lower specific gravity (0.3–0.5) and physical properties intermediate between kabur (*Dryobalanops* sp.) and white, yellow or red seraya (Ong 1985). A study on a hybrid from Ulu Kukut was carried out by Sining Unchi (1991). According to this study, the wood of the hybrid has slightly better properties than the wood of *A. mangium* but, given the wide range of variation within samples of both the hybrid and the pure species, there is a frequent overlapping of the different trait ranges.

Other properties

The pulping qualities of the species range from good to very good (Clark et al. 1991), *A. auriculiformis* having a higher pulpwood productivity (234–305 kg/m^3) than *A. mangium* (179–268 kg/m^3). These results are as good as, and often better than, those obtained for several *Eucalyptus* spp.

The use of *A. mangium* as a source of raw material for medium density fibreboard has been described by Chew and Jaffar Ahmad (1986), Chew et al. (1990), Rahim et al. (pers. comm.) and others. These studies show that *A. mangium* is a potential source of raw material for medium density fibreboard but this needs to be confirmed by large-scale experiments.

There are no conclusive studies of the pulping properties of the hybrid or of its use as raw material for fibreboard, but it is reasonable to believe that it will perform as well as *A. mangium*, and probably better given its higher wood density.

Site adaptability

Although there is no formal experimentation on the site adaptability of the hybrid, some interesting conclusions may be drawn from an article by Rufelds and Lapongan (1986) and observations by several people such as Wong Chin Yong and Sim Boon Liang in charge of different research programs.

- The hybrid has been found in all the existing *A. mangium* plantations and performs at least as well as the pure species in most of the cases. It appears, therefore, that the hybrid possesses the same site adaptability as *A. mangium*.
- In view of the high adaptability of *A. auriculiformis*, it seems possible to produce some hybrid populations with a wide range of site adaptability (semi-dry areas, saline areas, etc.) by using controlled crosses between selected parent populations.

Prospects of the Hybrid

As we have seen, the hybrid offers basically the same fields of utilisation as the pure species. It shows a better stem straightness than *A. auriculiformis* and better stem circularity and self-pruning ability than *A. mangium*. Its growth performances, in Sabah, are as good as those of *A. mangium* and there is a possible heterosis depending on sites. The wood characteristics are intermediate between *A. mangium* and *A. auriculiformis*, and the hybrid can be used as a suitable substitute for these two species. In the case of *A. mangium*, the hybrid is probably of better quality for plywood, fibreboard and pulpwood, mainly because of its higher basic density.

Another interesting field of activity for the use of the hybrid could be the development of disease-resistant lines. To date, it seems that *A. auriculiformis* is free of heart-rot disease, whereas *A. mangium* can be severely attacked. Heart-rot resistance in *A. mangium* could perhaps be induced by means of the hybridisation.

There are, therefore, various bright prospects for the use of *A. mangium* × *A. auriculiformis* in plantations provided that:

- a suitable, practical and cheap technique of controlled pollination can be developed;
- good vegetative propagation techniques are available;
- the hybrid trees are produced by crosses between elite trees of the two pure species, which implies setting up a soundly based breeding program for both *A. mangium* and *A. auriculiformis*.

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A Review of Provenance Variation in Growth of *Acacia mangium*

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Abstract

This paper reviews FAO-coordinated international provenance trials of *Acacia mangium* established in the 1980s. Data from a total of 19 trial sites in Southeast and South Asia, Australia and Fiji were studied. A total of 24 local provenances from five provenance regions, including two provenances from Papua New Guinea (PNG), 19 from Queensland Cairns Region (QCR), and one each from Far North Queensland (FNQ), Ceram (CERAM) and Irian Jaya (IRIAN) were tested. None of the trials tested all 24 provenances, and the subsets tested varied from site to site. Analysis of variance of height and/or diameter at breast height (dbh) for individual trial sites showed significant differences among provenances tested at every trial site.

Height and dbh were converted to mean annual increments in height and dbh for an analysis of variance across trial sites. There were highly significant differences in performance between experimental sites, between provenance regions, and among the local provenances within provenance regions. Growth was generally faster at near-equatorial trial sites, with mean annual increment in height around 3–4 m, and slower at sites further from the equator in Bangladesh, south China, Taiwan and Fiji. PNG provenances were consistently the best performers, closely followed by the Claudie River provenance from FNQ. Provenances from the QCR provenance region (16–18°30'S) and the CERAM and IRIAN provenances were almost always slower-growing than those from PNG and FNQ.

Acacia mangium Willd. has become an important plantation species in the humid tropics over the last two decades. Seed collections for international provenance trials were coordinated by CSIRO and FAO in the early 1980s (Doran and Skelton 1982; Turnbull et al. 1983). A number of published provenance trials (Atipanumpai 1989; Chung et al. 1990; Hadi et al. 1990) have shown substantial differences in the growth performance of different natural provenances. Inquiries were made by the Australian Tree Seed Centre in 1990 to obtain the latest data sets from all known trials of the FAO–CSIRO seedlots. The replies received included several with previously unpublished data, enabling an analysis of growth performance of provenances across a wide range of tropical sites.

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Materials and Methods

Provenances tested

Details of the *A. mangium* provenances included in the trials are provided in Table 1. Estimates of key climatic parameters for provenance locations in Queensland and Papua New Guinea (PNG), calculated using the BIOCLIM computer program (Booth et al. 1988), are presented in Table 2.

Seedlots tested were all bulks from at least five well-spaced parent trees (10 or more parents in most cases), and may be considered as representing local provenances. They can logically be assigned among five geographically separate provenance regions. The term provenance region is used here to denote a broad geographic area of natural occurrence, and does not imply

Table 1. List of provenances included in *A. mangium* international provenance trials.

CSIRO no.[1]	Provenance region[2]	Lat. (°S)	Long. (°E)	Alt. (m)	#par.[3]	Location
12990	QCR	16 34	145 35	400	10	Julatten
12991	QCR	16 17	145 31	60	10	Daintree
12992	QCR	16 30	145 32	30	10	Rex Range
13229	FNQ	12 44	143 13	60	6	Claudie River
13230	QCR	17 53	146 06	5	10	Mission Beach
13231	QCR	17 42	145 57	40	5	NW of Silkwood
13232	QCR	17 41	146 05	5	10	Cowley Beach
13233	QCR	17 06	145 48	20	10	NE Walshs Pyramid
13234	QCR	17 02	145 48	20	10	E of Cairns
13235	QCR	17 35	146 05	20	5	Mourilyan Bay
13236	QCR	17 46	146 05	10	5	Kurrimine
13237	QCR	17 50	146 01	20	10	El Arish
13238	QCR	17 56	146 02	70	10	Mission Beach
13239	QCR	17 55	145 52	50	10	Tully
13240	QCR	18 14	145 50	60	5	Cardwell-Ellerbeck
13241	QCR	18 21	146 03	50	5	Broken Pole Creek
13242	QCR	18 26	146 01	60	10	Abergowrie S.F.
13279	QCR	16 17	145 31	60	25	Daintree
13459	PNG	8 45	141 25	30	20	Morehead
13460	PNG	8 50	143 08	10	18	Oriomo River
13534	QCR	16 32	145 25	60	70	Cassowary Range
13621	CERAM	3 04	128 12	150	99	Piru, Ceram
13622	IRIAN	0 46	133 34	30	15	Sidei
13846	QCR	16 31	145 24	60	75	Mossman

[1] Seedlot identifier number used by CSIRO's Australian Tree Seed Centre

[2] Provenance regions: QCR = Queensland Cairns region
FNQ = far north Queensland
PNG = Papua New Guinea
IRIAN = Irian Jaya, Indonesia
CERAM = Ceram, Indonesia

[3] Number of parent trees represented in provenance seedlot

that local provenances from within the provenance region will necessarily have similar genetic characteristics (Turnbull and Griffin 1986). The provenance regions are as follow:

Queensland Cairns Region (QCR) — the southern occurrences of *A. mangium* along the east coast of Queensland, Australia, south of latitude 15°S. In this provenance region there are many small to medium sized populations marginal to rainforest, occurring over 3 degrees of latitude to around 18°30'S and from sea level up to about 400 m altitude. Nineteen seedlots were included in the trials.

Far North Queensland (FNQ) north of latitude 13°S. Limited occurrences on the margins of rainforest along rivers flowing to the eastern and western sides of Cape York, including the Claudie, Pascoe, Wenlock, Olive and Jardine Rivers. A single six-tree collection from Claudie River was tested in the trials.

Papua New Guinea (PNG) — *A. mangium* extends across the southern lowlands of Western Province, Papua New Guinea. It is a common species here, occurring locally in populations of varying size on better drained sites. The Western Province populations are contiguous with extensive populations in south-eastern Irian Jaya (Gunn and McDonald 1991). The two PNG seedlots used in the trials were separated from one another by about 180 km.

Ceram, Indonesia (CERAM) — *A. mangium* occurs on the island of Ceram in Indonesia. A small population near Piru was sampled (Turnbull et al. 1983).

Irian Jaya, Indonesia (IRIAN) — A single, small (15 ha) population at Sidei on the Vogelkop Peninsula of Irian Jaya in Indonesia (Turnbull et al. 1983).

Trial sites

Information on the trial sites is given in Table 3. Data was available from sites in Borneo (Sabah, Sarawak and South Kalimantan), Sumatra, Thailand, southern China, Taiwan, Bangladesh, Melville Island in the far north of Australia, and Fiji. It is hoped to include data from trials in Central America, Peninsular Malaysia, the Philippines, the Solomon Islands and West Africa in a more comprehensive analysis to be published later.

Table 2. BIOCLIM estimates of climatic parameters for provenance locations in Queensland and Papua New Guinea.

CSIRO no.	Lat. (°S)	Long. (°E)	Alt. (m)	MAT[1] (°C)	MMTHM[2] (°C)	MMTCM[3] (°C)	MAP[4] (mm)	MPDQ[5] (mm)
PNG								
13459	8 45	141 18	30	26.2	32.3	20.8	1764	102
13460	8 50	143 08	10	26.3	32.3	20.9	2090	184
FNQ								
13229	12 44	143 13	60	26.0	33.3	18.8	1876	50
QCR								
12990	16 34	145 35	400	23.0	32.5	12.3	2002	123
12992	16 30	145 22	30	25.1	31.8	17.4	2128	96
13230	17 53	146 06	5	23.8	31.6	14.4	3156	225
13231	17 42	145 57	40	23.6	31.6	14.2	3601	281
13232	17 41	146 05	5	23.8	31.5	14.8	3258	246
13233	17 06	145 48	20	24.3	31.4	16.2	2161	80
13234	17 02	145 48	20	24.4	31.3	16.5	2162	107
13235	17 35	146 05	20	23.7	31.4	14.8	3243	249
13236	17 46	146 05	10	23.8	31.5	14.5	3277	247
13237	17 50	146 01	20	23.7	31.6	14.2	3411	268
13238	17 56	146 02	70	23.8	31.8	14.0	2061	236
13239	17 55	145 52	50	23.7	31.9	13.8	3678	338
13240	18 14	145 58	60	23.0	32.1	13.6	2061	75
13241	18 21	146 03	50	23.8	32.2	13.3	2061	96
13242	18 26	146 01	60	23.9	32.3	13.1	1951	89
13279	16 17	145 31	60	25.2	31.7	18.0	2781	185
13846	16 31	145 24	60	25.1	31.9	17.4	1977	86

[1] MAT = mean annual temperature
[2] MMTHM = mean maximum temperature of hottest month
[3] MMTCM = mean minimum temperature of coldest month
[4] MAP = mean annual precipitation
[5] MPDQ = mean precipitation of driest quarter

Climatic data for CERAM and IRIAN could not be estimated

Traits examined, and methods used to examine data

Height and/or diameter at breast height (dbh) at a known age were available from all trial sites (height was not measured at two of the sites and dbh not measured at one). For trees that had more than one stem at breast height, dbh was in some trials the dbh of the largest stem, while in others it was the dbh equivalent to the tree's total cross-sectional area at breast height. Percentage survival was available for most sites and, for a few sites, summary data on stem form were also available.

All the trials reported here used replicated randomised complete block designs, although the size and shape of plots and the number of replications varied. Analysis of variance of height and dbh for each individual trial site was carried out either by the organisation managing the trial, or in the course of this study. There were statistically significant differences among provenances for height and/or dbh at every trial site.

Table 4 shows which seedlots were tested at each trial site. In addition to the different combinations of seedlots tested, there were differences between sites in silvicultural techniques, such as rates of fertilizer application and weeding schedules, which undoubtedly affected growth rates. These factors make comparisons of the growth potentials of different sites only approximate.

Age at the time of measuring varied from 1.5 to 7 years, but was around 3 to 4.5 years in most cases. Examination of time series growth data from the trials at Taiwan (2.5, 3.5 and 4.5 years) and Barito, South Sumatra (2 and 7 years) indicated that the annual increments in height and dbh were constant over the first 2–7 years after planting at these sites. This suggested that comparisons between sites of mean annual increments (MAIs) would be little affected by the between-site differences in age of measurement. MAI in height and diameter was calculated for each provenance at each trial site by dividing height and diameter by the age at the time of measurement, and this data was further analysed.

Table 3. Trial sites included in the analysis.

(a) Site code, location, organisation conducting trial and information source

1 SbA, SbB, SbC, SbD
Sipitang, Sabah, Malaysia (4 trial sites)
Sabah Forest Industries. Sim Boon Liang (pers. comm.).

2 Sfda
Bengkoka, Sabah, Malaysia
SAFODA. Miller and Hepburn (1989)

3 Swk1, Swk2, Swk3, Swk4, Swk 5
Sarawak, Malaysia (5 trial sites)
Forest Department, Sibu, Sarawak
Forest Silviculturalist (pers. comm.).

4 Skl
South Kalimantan, Indonesia
Enso/Indonesian Ministry of Forests. Hadi et al. (1990)

5 Sum
South Sumatra, Indonesia
Barito Group. P. Havmoller (pers. comm.).

6 Thai
Lad-Krating, Thailand
Kasetsart University. Atipanumpai (1989)

7 Chi L, Chi H
Southern China (2 trial sites, Leilin and Haikang)
Chinese Academy of Forestry. Pan Zhi-Gang (pers. comm.).

8 Taiw
Chung-Pu, Taiwan
Taiwan Forestry Research Centre. Chung et al. (1990).

9 Melv
Melville Island, Northern Territory, Australia
Conservation Commission of the Northern Territory
M.W. Haines (pers. comm.).

10 Fiji
Nukurua, Fiji
Fiji Ministry of Forests. L.R. Jiko (pers. comm.).

11 Bdsh
Keochia, Bangladesh
Bangladesh Forest Research Institute.
Zashimuddin et al. (1985).

(b) Site data

Site	Lat.	Long.	Alt. (m)	MAT[1] (°C)	MAP[2] (mm)
SbA	5° N	115°30 E	100	26	3100
SbB	n.a.*	n.a.	500	24	3100
SbC	n.a.	n.a.	650	23	3200
Sbd	n.a.	n.a.	1300	20	2000
Sfda	6°55 N	117°05 E	n.a.	26	2600
Sw1	1°03 N	109°55 E	35	26	n.a.
Sw2	2°13 N	111°30 E	30	26	n.a.
Sw3	2°17 N	112°00 E	30	26	n.a.
Sw4	3°22 N	113°37 E	35	26	n.a.
Sw5	3°45 N	113°49 E	170	26	n.a.
Skl†	3°S	115° E	20	26	2100
Sum	3°45 S	103°55 E	75	26	2600
Thai	13°42 N	101°06 E	80	28	1220
Chi L	20°38 N	110°31 E	50	22.6	1473
Chi H	20°55 N	110°06 E	n.a.	22.9	1701
Taiw	22°23 N	120°31 E	250	22.1	3056
Melv	11°34 S	130°34 E	20	25	1750
Fiji	17°50 S	178°20 E	50	25	3000
Bdsh	22°06 N	92°05 E	n.a.	24	2590

[1] MAT = mean annual temperature
[2] MAP = mean annual precipitation
* n.a. = not available
† Approximate values only

Because eight of the provenances in Table 1 were missing from all the sites on the island of Borneo, and another five provenances present at only one of the Borneo sites, it was decided to partition the analysis into two separate analyses, one for the eleven Borneo sites, and another for the remaining eight sites in the other countries where there was a fuller representation of provenances. The two analyses of variance looked at variation between sites, provenance regions, provenances within provenance regions (i.e. the 13 provenances in the QCR provenance

Table 4. Seedlots tested at each trial site.

	SbA	SbB	SbC	SbD	Sfda	Sw1	Sw2	Sw3	Sw4	Sw5	SKI	Sum	Thai	Chi L	Chi H	Taiw	Melv	Fiji	Bdsh
12990														1		1			1
12991	1	1	1	1															
12992	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1		1
13229	1	1	1	1		1					1	1	1	1	1	1	1		
13230														1		1			
13231															1		1	1	
13232	1		1	1	1								1	1	1	1			
13233	1		1	1	1		1						1	1	1	1		1	1
13234													1	1	1	1	1	1	
13235					1								1	1		1		1	1
13236													1	1		1			
13237													1	1		1			
13238	1	1	1	1	1							1	1	1		1		1	1
13239													1	1		1		1	
13240					1								1	1		1	1	1	
13241	1	1	1	1	1	1	1	1	1	1			1	1		1		1	1
13242					1							1	1	1	1	1			1
13279														1		1			1
13459												1	1	1	1	1	1		1
13460					1								1	1		1	1	1	1
13534	1	1	1	1		1	1	1	1	1		1							
13621	1	1	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1
13622	1	1	1	1		1	1	1	1	1	1		1	1		1	1	1	
13846						1												1	

1 = tested

region, and the two provenances in the PNG provenance region), and the interaction between sites and provenance regions.

Results

Survival

Survival rates at the time of measurement were high at most sites, as summarised in Table 5.

Analysis of variance across sites and provenances

For both height MAI and dbh MAI, and for both the Borneo and 'non-Borneo' analyses, there were highly significant differences ($P<0.001$) between sites, between provenance regions and between provenances within provenance regions. The interaction of sites and provenance regions was also highly significant ($P<0.001$) for the Borneo sites but only barely significant ($P<0.05$) for height MAI and not significant for dbh MAI for the 'non-Borneo' sites. The analyses of variance will be reported in more detail in a later publication.

Table 5. Survival rates of *Acacia mangium* at trial sites.

Trial site	Age at which trial assessed (years)	Range of survival for individual provenances (%)
Sabah Forest Industries		
Site A	4.0	70–86
Site B	4.0	64–91
Site C	3.0	70–78
Site D	3.0	70–85
Safoda, Sabah	4.0	n.a.*
Sarawak (5 sites)	4.0	n.a.
South Kalimantan	2.5	100
South Sumatra	7.0	60–72
Thailand	2.5	98–100
South China (2 sites)	4.0	n.a.
Taiwan	4.5	n.a.
Melville Island, Australia	4.5	75–98
Fiji	3.0	17–77
Bangladesh	1.5	32–80

*n.a. = not available

Summary of performance of provenance regions across trial sites

The performance of the five provenance regions at the different trial sites is summarised in Table 6, and illustrated for the 'non-Borneo' sites in Figs 1 and 2. The MAIs shown for QCR and PNG provenance regions are the averages of the individual seedlots from those provenance regions tested at each site. As different sub-groupings of provenances from QCR and PNG were tested at different sites, the comparisons across sites are not exact.

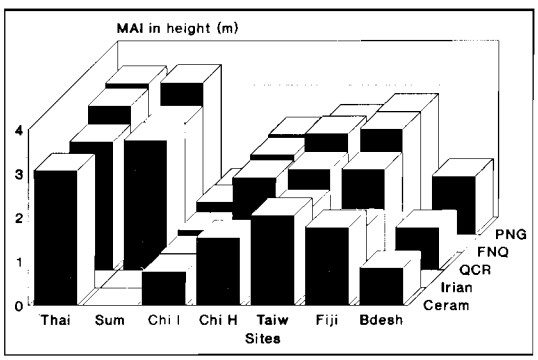

Fig. 1. Mean annual increment in height of provenance regions for trial sites other than those in Borneo (see Table 3 for site codes).

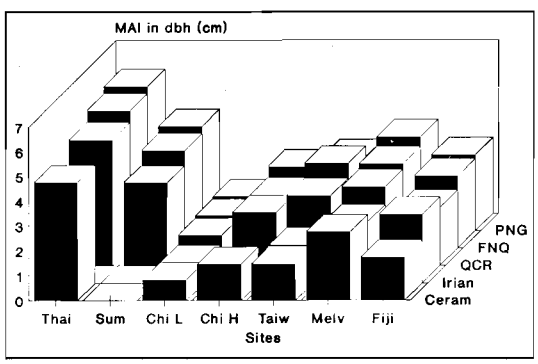

Fig. 2. Mean annual increment in dbh of provenance regions for trial sites other than those in Borneo (see Table 3 for site codes).

There was a clear trend of more rapid growth at sites close to the equator, with height MAI of around 4 m at some sites. Growth at sites close to the Tropic of Capricorn in south China, Taiwan and Bangladesh, and at Fiji (17°50'S), was much slower, with height MAI of only around 1–2 m. In south China, the slower growth at Leilin relative to Haikang is attributable to lower soil fertility at Leilin (Pan Zhi-gang, pers. comm.).

A consistent difference in the performance of the provenance regions is also apparent. PNG was almost always the best provenance region where it was tested, although it was only included at one Borneo trial site. The single FNQ provenance also performed well everywhere it was included, but with slightly slower growth than PNG at most locations. These trends held for both height and diameter increment, so the advantage in volume production of PNG and FNQ over the other three provenance regions was substantial in most cases. Results of a provenance trial in Ivory Coast, West Africa, summarised by Souvannavong (1990) are in accordance with the general trend of superior growth performance of PNG and FNQ relative to the other three regions. Preliminary provenance rankings from four trial sites in Peninsular Malaysia (Baharudin and Chew 1987) also show the same ranking of provenance regions for height and dbh.

At the trial sites in Taiwan and south China, one or two individual QCR provenances grew faster than PNG and FNQ, but the differences were not significant. CSIRO nos 13237 and 13239 were the best QCR provenances in Taiwan, while 13242 was best at both sites in south China. CSIRO no. 13242 also performed as well as the two PNG provenances in Bangladesh. At Sabah Forest Industries sites A and B, the FNQ provenance was outperformed by the CERAM seedlot and one or two QCR provenances, but again these differences were not significant. At every other trial site where they were included, PNG and FNQ outperformed CERAM, IRIAN and all QCR provenances. It must be kept in mind, however, that the FNQ provenance region is only represented in these trials by a single six-tree seedlot.

The relative performance of QCR, CERAM and IRIAN was variable, with generally better performance of CERAM and IRIAN at sites closer to the equator, while QCR provenances outperformed them in south China, Taiwan, Bangladesh, Fiji and Melville Island, Australia.

Form

Form assessments are not yet available for many of the trial sites, so an analysis of site and provenance differences in form has not been attempted at this stage. The percentages of trees with single stems at two trial sites in Sabah, the site at Melville Island, Australia, and the trial in Ivory Coast, West Africa (Souvannavong 1990), are tabulated in Table 7 to illustrate the range of variation in form among provenances and the substantial differences that occur between sites.

Discussion

It is clear from the results of the trials reported here, and other subsequent provenance trials, that there must be substantial genetic differences between provenance

Table 6. Mean annual increment (MAI) in height and dbh: performance of provenance regions and overall site mean at each trial site.

Trial site	Height MAI (m)						Dbh MAI (cm)					
	CERAM	IRIAN	QCR	FNQ	PNG	MEAN*	CERAM	IRIAN	QCR	FNQ	PNG	MEAN*
SbA	3.80	3.60	3.13	3.64		3.30	3.35	3.10	2.78	3.30		2.92
SbB							2.50	2.38	2.16	2.29		2.23
SbC	4.15	3.99	3.92	4.19	4.22	3.98	4.04	3.80	3.91	4.46	4.30†	3.97
SbD	3.52	3.31	3.44	3.69		3.46	3.80	3.37	3.76	4.48		3.79
Sfoda		2.68	2.98		3.27	2.98		2.85	2.96		3.23	2.98
Swk1	1.92	1.88	1.42	2.17		1.63	1.62	2.36	1.85	2.83		2.01
Swk2	2.10	3.08	2.74			2.68	1.55	3.07	2.83			2.62
Swk3	1.80	2.13	2.11			2.05	1.70	2.13	2.33			2.16
Swk4	2.71	3.21	3.32			3.18	2.53	3.21	3.47			3.23
Swk5	3.43	3.52	3.34			3.40	2.93	3.54	3.19			3.21
Skl	3.62	3.58	4.44			3.88	3.26	3.29	4.64			3.73
Sum			2.96	2.93	3.46	3.05			3.35	3.96	4.23	3.65
Thai	3.06		2.92	3.33	3.44	3.02	4.77		5.09	5.56	5.84	5.20
Chi L	0.76	0.78	0.91	1.14	1.12	0.93	0.84	0.83	1.22	1.31	1.39	1.20
Chi H	1.54		2.10	2.22	2.28	2.07	1.46		2.15	2.15	2.60	2.12
Taiw	2.04	1.83	2.29	2.70	2.56	2.30	1.48	1.54	2.83	3.44	3.01	2.75
Melv							2.76	2.47	3.19	3.42	3.80	3.23
Fiji	1.76	1.39	2.29	2.80	2.81	2.26	1.73	1.19	2.34	2.91	3.07	2.31
Bdsh	0.84		0.96		1.33	1.02						

* Mean of all local provenances tested at site

† PNG seed source, but not one of the two listed in Table 1. Not included in computation of site mean or across-sites ANOVA

Table 7. Percentage of trees with single stems at three trial sites, by provenance.

CSIRO seedlot no.	Provenance		Trial site			
	Region	Name	Sabah SbC[1]	Sabah SbD[1]	Melville Is. Australia[2]	Ivory Coast[3]
12991	QCR	Daintree	34	18	–	–
12992	QCR	Rex Range	51	13	41	–
13229	FNQ	Claudie River	50	24	11	89
13232	QCR	Cowley Beach	44	22	–	–
13233	QCR	Walshs Pyramid	47	26	–	84
13234	QCR	E. of Cairns			17	
13238	QCR	Mission Beach	48	17	–	
13240	QCR	Cardwell-Ellerbeck			10	77
13241	QCR	Broken Pole Ck	45	33	–	76
13459	PNG	Morehead	–	–	22	
13460	PNG	Oriomo River	–	–	12	75
13534	QCR	Cassowary Range	51	37	–	–
13864	QCR	Mossman				92
13621	CERAM	Piru, Ceram	42	23	1	56
13622	IRIAN	Sidei	66	33	12	69
–	PNG	n.a.*	47	–	–	–
–	Sabah	(from plantation, Lhad Datu)	68	28	–	–

[1] Sim Boon Liang (pers. comm.). Percentage of trees with single stems.

[2] M.W. Haines (pers. comm.). Percentage of trees with single stems at 3.0 m height.

[3] Souvannavong (1990). Percentage of trees with single stems.

* not available.

regions and local provenances, because of the consistent and significant differences in their growth performance. Isozyme analysis indicates that *A. mangium* has low genetic diversity (Moran et al. 1989). Isozyme studies only examine a small sub-set of the genome, and therefore do not provide an absolute measure of overall genetic variation. However, it may be noted that the two Indonesian provenances had the lowest isozyme diversity and the poorest performance in the provenance trials. Their poor performance might well be due to inbreeding in populations derived from a narrow genetic base, perhaps founded from only one bird-transported seed or possibly by human introduction.

The growth rate of PNG provenances remained higher than that of most QCR provenances at trial sites with cooler winter climates in Taiwan and south China. Slower growth of PNG relative to QCR at these sites might be anticipated on the grounds that QCR provenances would be better adapted, because they originate from climates with cooler winters (Table 2). While a few QCR provenances did outperform PNG and FNQ in China and Taiwan, differences were not significant and, as noted above, the interaction between provenance regions and experimental sites for the 'non-Borneo' sites was only barely significant for height MAI and not significant for diameter MAI.

It was noteworthy that growth at Sabah Forest Industries trial site D, altitude 1000 m and estimated mean temperature of 20°C, was much faster (height MAI 3.46 m) than growth at the Bangladesh, south China and Taiwan sites where mean annual temperatures are in the range 22–24°C, but height MAIs were only 1–2 m. It appears that low winter temperatures halt growth for several winter months at subtropical sites (Yuo Yintian 1989), while growth continues year-round in the mild conditions at the high-altitude equatorial site in Sabah where there is little seasonal variation in temperature. However, poor apical dominance at the high-altitude Sabah site results in trees of poor form with multiple stems (Table 7; Sim Boon Liang, pers. comm.). In Thailand, periods of low diurnal temperature range are associated with rapid diameter growth, although it is hard to separate this effect from seasonal variations in rainfall (Atipanumpai 1989).

Since the international trials reported here were conducted, seed collections for further research, including single-tree collections from a total of several hundred parent trees, have been undertaken by the Australian Tree Seed Centre in many natural provenances of *A. mangium*, particularly in the PNG, FNQ and QCR provenance regions (Gunn and Midgley 1991; Gunn and McDonald 1991). Other provenance trials have been planted, and the individual tree collections have been used to establish the base populations of a number of breeding programs. However, nothing is known to date about the genetic gains in growth and form that may be obtainable by crossing between different provenance regions of *A. mangium*.

Acknowledgments

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Studies on Widespread Attack of Ambrosia Beetle, *Platypus* sp., in Provenance Trial Plots of *Acacia crassicarpa* in Sipitang District of Sabah, Malaysia

R.S. Thapa*

Abstract

Widespread infestation by the ambrosia beetle (*Platypus* sp.), has been recorded in all the provenance trial plots of *Acacia crassicarpa* planted during the period 1985–1988 in the concession area of Sabah Forest Industries Sdn Bhd in Sipitang District of Sabah, Malaysia. Sampling of trees in these plots showed that 27–86% trees were lightly to heavily attacked. It is concluded that the borer, which is endemic to the natural forest of Sabah, has now switched to plantations of exotic species.

Observations show that the borer has a life cycle of 5–6 weeks. Ten to 15 days after the initiation of boring, black-stained sap starts oozing out of the holes. In heavily attacked trees, black stain from the tunnels spreads out in the sapwood region of the bole to a considerable distance depending upon the severity of attack. The black staining of the sapwood is caused by bacterial infection transmitted by the borer. The pathogenicity of the bacterial infection has yet to be studied.

No apparently deleterious effect of heavy borer attack has so far been visible on the trees; but it is feared that several bored holes in the basal region of the tree trunk, and the development of black sap stain due to bacterial infection in the major portion of the sapwood of the bole, may considerably reduce the vitality of the tree and expose it to attack by other biodegrading agencies.

AMBROSIA beetles (Platypodinae) are known to attack commercial trees in natural forests of Sabah (Thapa 1971). They are also potential pests of young plantations (Sajap et al. 1990). The adult beetles bore small holes (1–3 mm), and carry fungi, yeast and bacteria in specialised structures called 'mycangia' located on the head or thorax. Fungi are cultivated on the walls of bored tunnels or in specialised beds to serve as food. Bacteria transmitted by the beetles may cause stains in trees (Anon. 1986).

In 1989 widespread attack of the ambrosia beetle, *Platypus* sp., occurred in all the provenance trial plots of *Acacia crassicarpa* planted during 1985–1988 in the concession area of Sabah Forest Industries Sdn Bhd in Sipitang District of Sabah, Malaysia. Initially a few holes may be bored in a tree, but subsequent attacks compound the damage.

During February–June 1991, studies were carried out on the biology of the borer, incidence of attack in all the trial plots, development of black stain in attacked trees subsequent to borer attack due to bacterial infection, and the impact of borer attack on trees. The results of these studies are presented in this paper.

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Biology of the Ambrosia Beetle, *Platypus* sp.

The beetle is about 3.5 mm in length and 1.00 mm in diameter. Field observations show that the life cycle of the borer is completed within 5–6 weeks. As the beetle starts boring, wood-dust is ejected out of the tunnel. This wood-dust may form a compact mass protruding from the hole as a short stick, never exceeding 0.3 mm in length, before breaking off and falling to the ground. In the case of freshly attacked trees with a few hundred holes at the basal portion of the tree trunk, wood-dust may be seen accumulated on the ground at the base of tree. Ten to 15 days after initiation of the boring, exudation of black-stained sap follows and, when it dries out, the entrance to the hole may get clogged.

The fungal spores carried by the beetles are deposited on the walls of the tunnels for culturing. Egg-cradles may also be cut on the walls of the tunnel by the female beetle for egg-laying. The larvae, on hatching from the

eggs, feed on the fungal mycelia and spores being cultivated in the tunnel. On maturity, the adult beetle emerges from the same hole bored by the parent beetle by boring through the solidified sap at the entrance to the hole. As soon as the adult beetles emerge, they search the host tree for fresh boring. The flight period of the beetles is thus very short.

Fresh borer holes may be recognised by wood-dust ejection and the subsequent exudation of black-stained sap. Old borer holes are distinguished by a black patch formed by dried sap exudate.

Incidence of Borer Attack in Provenance Trial Plots

Ambrosia beetle attack was first discovered in April 1989 in all the provenance trial plots of *Acacia crassicarpa* in Compartments P3, L42B, L42A, L40, L39 and L21A. Observations then showed that 50–80% trees were infested. These plots are widely separated. Each plot has an area of 0.27 ha or slightly less, and is usually surrounded by plantations of *Acacia mangium*. The plot of *A. crassicarpa* in Compartments P3, L42A, L39 and L21A is intermixed with *Acacia auriculiformis* and *A. aulacocarpa* (Plate 1).

During February–May 1991, an assessment of borer attack in all the trial plots was carried out. In each plot 70–200 trees were sampled. For sampling, 2–3 lines of trees were taken at different points. Though borer holes were scattered over the whole length of the tree bole, they were concentrated on the basal portion up to a height of about 2 m and only the holes over this length were counted in assessing degree of borer attack. Each hole was marked with white paint so that subsequent holes could be detected (Plate 2). After initial counting of the holes in each tree, observations on the progress of borer attack were followed at 10–15 days interval. The degree of attack in each tree was categorised arbitrarily as light with 11–30 holes, moderate with 31–50 holes, and heavy with more than 50 holes. Less than 11 holes was categorised as negligible and not taken into account unless the number subsequently increased due to repeated attacks.

The present status of borer attack in all the trial plots is:

1. Compartment P3, Lumaku Highland (ca 1000 m. a.s.l.). Planted in July, 1986.

   Some 102 trees were sampled in April 1991. Of these, 23% had been attacked lightly and 47% moderately to heavily. Only one tree was freshly attacked with 130 holes, and ejected wood-dust had accumulated on the ground at the base of tree. All attacked trees ranged from 11 to 23 cm dbh. Trees less than 11 cm dbh were rarely attacked or, if attacked, had a negligible number of holes. The highest number of holes recorded in a tree was 245.

2. Compartment L42B (ca 500 m. a.s.l.). Planted in May 1988.

   Two hundred trees were sampled during February–March 1991. About 14% trees were attacked lightly and 46% moderately to heavily. dbh of attacked trees ranged between 11.6 and 27.6 cm. The highest number of holes in a tree was 297. Four trees were found freshly attacked, one in February and three in April 1991, while the rest had old borer holes with a distinct black patch around them (Plate 3). The freshly attacked trees had in total 72, 200, 211 and 297 holes respectively. Observations showed that black-stained sap exudation started from the holes within 10–15 days of initiation of boring, and continued to ooze for 4–5 days before drying up around the holes. However,in the case of four holes in one tree, and five holes in another tree, the exudate flowed down the bark forming a wide black streak 10–12 cm long. A second attack of a few borers occurred in all these freshly attacked trees in April 1991.

   To study the extent of the damage, two wind–uprooted trees, still green and heavily attacked, and one freshly felled tree, also heavily attacked, were examined by working out the bored holes. Each of these trees had more than 100 holes on its bole up to a height of 2 m and a number of holes were scattered over the bole up to a height of 8 m. The tunnels, unbranched and 5–6 cm in length, were bored deep into the heartwood, perpendicular to the wood surface. The wood around the tunnels was stained black. From the tunnels, streaks of black stain radiated upwards and downwards in the sapwood region (Plate 4) and, because of the many holes in the basal portion of the tree-trunk, streaks of one tunnel had joined with that of an adjacent one and formed an almost continuous and circular belt in the sapwood region. This could be seen when the basal portion of a heavily attacked tree was cut across.

3. Compartment L42A (ca 100 m. a.s.l.). Planted in December 1985.

   In all 72 trees were sampled in May 1991. Of these 22.2% trees were attacked lightly and 61.1% moderately to heavily, ranging in dbh between 12.6 and 21 cm. The maximum number of holes was 238. Four trees were freshly attacked with 2–8 holes in each.

4. Compartment L40 (ca 500 m. a.s.l.). Planted in July 1988.

   In all 100 trees were sampled in May 1991. Only 14% trees were attacked lightly and 13% moderately to heavily, ranging in dbh between 11 and 17 cm. The percentage of trees attacked was much less than in other compartments because the majority of trees were below the preferred minimum size for borer attack of 11 cm dbh. The maximum number of holes bored in a tree was 70.

Plate 1. A view of provenance trial plot of *Acacia crassicarpa* in Compartment L42B

Plate 2. A borer-attacked tree of *A. crassicarpa*. Borer holes are marked with white paint.

Plate 3. The distinct black patches surrounding borer holes.

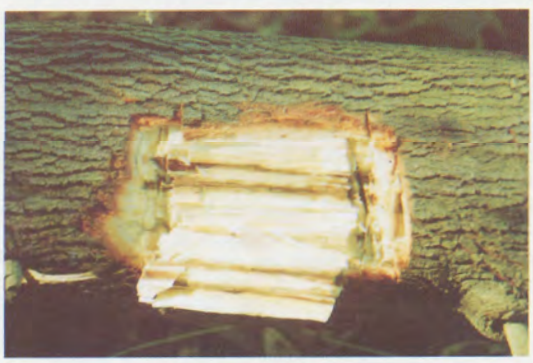

Plate 4. The black stains caused by bacterial infection in the sapwood region of a tree trunk.

5. Compartment L39 (ca 500 m a.s.l.). Planted in January 1986.

   Of a total of 100 trees sampled in February 1991, 13% of trees were attacked lightly and 72% moderately to heavily, ranging in dbh between 14 and 26 cm. Fresh attack was observed in only one tree in the last week of February 1991. This tree was again attacked with some 15 bored holes in April 1991. The maximum number of holes was 178.

6. Compartment L21A (ca 560 m a.s.l.). Planted in April 1986.

   Of the 104 trees sampled in May 1991, 27.8% were attacked lightly and 57.7% moderately to heavily. All attacked trees ranged in dbh between 13 and 25 cm. Fresh borer attack was recorded in six trees with a few holes in each. The maximum number of holes was 125.

Bacterial Infection and the Development of Black Stain in the Sapwood

The stained wood obtained from the attacked tree was sent to the Pathologist, Agriculture Research Station, Tuaran, who identified the causal agent of the stain to be a bacterium. Exudation of blackstained sap immediately after the completion of tunnelling suggests that the beetles are the vectors of a bacterial infection.

Ambrosia beetles also carry fungi and implant them in their tunnels for cultivation which results in the black staining of wood around the tunnels. However, in the case of the *A. crassicarpa* trees, the black stain from the tunnels spread out into the sapwood region and, depending on the intensity of attack, it covered a considerable length of the bole. Absence of black stains in the heartwood region obviously shows that the infection spread out through the live cells. It is yet to be studied whether the infection, once established in the sapwood region, can continue to develop and spread throughout the sapwood region of the tree. The pathogenicity of the bacterial infection has also to be studied.

Other Hosts of Ambrosia Beetle

In the natural forests of Sabah, sporadic infection by the ambrosia beetle (*Platypus* sp.) has been recorded in some *Shorea* spp., mainly the red seraya.

In plantations, some species of *Acacia* and *Eucalyptus* have been found susceptible to *Platypus* sp. In Acacias, in addition to *A. crassicarpa*, light to moderate attack has occurred in *A. mearnsii, A. auriculiformis* and *A. aulacocarpa* in trial plots in Compartments P3 and L39, and *A. mangium*, which is being planted on a large scale throughout Sabah, has been attacked lightly in certain localities. In eucalypts, *E. grandis* has been found highly susceptible in Trial Plot P3, the attack being so severe that resin exudations from the bored holes covered nearly three-fourths of the bole length with a thick coating. *E. urophylla* has also been found moderately susceptible in Trial Plot P3.

Conclusions

It may be concluded that the ambrosia beetle (*Platypus* sp.), which is endemic to the natural dipterocarp forest of Sabah, has now switched to exotic plant species and has increased to epidemic levels in plantations of certain highly susceptible species like *A. crassicarpa*. The preferred minimum size of tree for attack by the borer is 11 cm dbh.

Despite heavy attack by the borer with about 300 holes in the basal portion of the tree bole, no outward symptom of any deleterious effect on the tree has been seen. However, it is feared that so many holes in the basal portion of its bole, and the development of black stain in the sapwood region all along the bole length due to bacterial infection, may considerably reduce the vitality of a tree and expose it to other biodegrading agencies.

Stained wood obtained from attacked trees was also tested for its suitability in paper making. It showed that more bleaching agent than usual was required for the stained wood, but the quality of the paper was in no way affected.

Management Approach

Host selection by ambrosia beetles involves visual or olfactory means or both. It may be the production of volatile compounds by the host tree which initially attracts the beetles. Alighting on the host tree and initiating boring, the beetles release pheromone to attract their own kind towards the selected host. Thus the pheromone is considered to be the main cause of their mass aggregation and pheromone-baited traps have been used successfully in suppression programs for ambrosia beetles.

In the present case of infestation of *Acacia crassicarpa* by ambrosia beetles, only the trial plots have been affected. As such, it is not expedient to go into the process of identification of the pheromone and its synthetic production. However, to cope with the problem, some simple and practical management strategies are outlined below.

One of the essential aspects of the study of the life history of the ambrosia beetles is to find out the active period/s of the beetles, if any, for laying out the traps.

For mass-trapping of beetles, various attractants will be tested such as bark-extract of *A. crassicarpa*, ethyl alcohol, white objects, etc.

The beetles attempting to bore in trees will be caught in tangle-foot or otherwise and used to serve as a pheromone- baited trap.

Acknowledgement

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Reproduction Biology and Breeding

Phenology of *Acacia mangium* and *A. auriculiformis* in Australia and Malaysia

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Abstract

Trees of *Acacia mangium* and *A. auriculiformis* were observed in Australia and Sabah to compare the phenological cycles, and to obtain detailed information on spike structure and pod production. Fertility of both species was higher in Sabah than in Australia due to increased level of flowering and longer flowering period. Flowering times also differed between Australia and Sabah. This may be due to the more uniform climate of the latter providing conditions suitable for flowering over a longer period of the year. The flowering periods in December–March and May–September in Sabah were those most likely to produce a pod crop in *A. mangium* and *A. auriculiformis* respectively. There was variability between the populations within the species in the mean spike length and number of flowers per spike. This requires further investigation to determine if this variability is due to hybridisation and introgression.

Acacia mangium Willd. and *A. auriculiformis* A. Cunn. ex Benth. are both native to northern Australia but are now widely cultivated for timber production outside their natural range. Malaysian interest in plantation establishment based on the two species, and on the interspecific hybrid between the two, has stimulated interest in their flowering and fruiting times. This is important for the scheduling of seed collection to coincide with peak pod production. It is commonly observed that species cultivated outside their natural distribution may have variable flowering and fruiting phenologies. For this reason we have studied the natural phenological cycle in northern Australia and compared this with the patterns observed in Malaysia. Of particular interest is the timing of flowering and fruiting, both within the species and in relation to overlap in flowering for hybrid seed production. We have also studied detailed aspects of spike structure and pod development of the two species in Australia and Malaysia to provide further information on their fertility.

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Materials and Methods

Plant material

Trees of *A. mangium* and *A. auriculiformis* were observed at a number of sites in Australia, and in Sabah, Malaysia. The observation site in Australia was Atherton in north Queensland (latitude 17°S, longitude 145°30'E). Those in Sabah were Karamunting and Sepilok, both near Sandakan (latitude 6°N, longitude 118°E); near Tawau (latitude 4°N, longitude 118°E); and at Bangawan and Mandahan, both near Kota Kinabalu (latitude 6°30'N, longitude 116°30'E).

Phenology studies

Trees of both species were selected and labelled, and were observed at fortnightly intervals for a two-year period, during 1987 to 1989 in Malaysia, and 1989 to 1991 in Australia. Details of the experimental trees are shown in Table 1. At each observation, the strength of flowering and fruiting was assigned visually to one of the following

Table 1. Experimental trees used in the phenology studies.

Location	Number of trees observed	Origin of seed	Age of trees at start of observations	Spacing (m)	Seed harvested
Acacia mangium					
Sepilok	5	Unknown, probably Ulu Kukut	14	8	No
Tawau 143G1	5	Mission Beach, Queensland	6	3	Yes
Tawau 74B2	5	Unknown	11	9	Yes
Tawau 35F1	5	Sabah (Forestry Dept Sook Plain, West Coast)	8.5	20	Yes
Tawau 92D	5	Sabah (SAFODA)	7	9	Yes
Tawau 69A	5	Mission Beach, Queensland plus three generations in Sabah	10.5	10	Yes
Atherton	10	Cape York	5–12	5–80	No
Acacia auriculiformis					
Karamunting	5	Unknown	Unknown, one old tree plus young progeny	8–30	No
Tawau 106G	5	Unknown	10.5	3	No
Tawau 13F	5	PNG	2.5	3	No
Tawau 85E	5	Unknown	10	3	No
Tawau 24H	5	Unknown	5	3	No
Tawau Arboretum	5	India	6	3	No
Atherton	10	Cape York	5–6	3.5–10	No

categories: 0 — none; 1 — light; 2 — medium; 3 — heavy. Numerical data corresponding to the visual assessments were obtained at the end of the phenology observation periods at Sepilok and Karamunting. Three representative branches were removed from each tree at each of the flowering categories 1, 2 and 3. All spikes were counted on each branch, and the total number of branches on each tree was recorded. A correlation curve relating the assessment category to the actual number of spikes was fitted for each species. Climate data recorded at the nearest weather station to each of the experimental sites is listed in Tables 2, 3 and 4.

Detailed flowering studies

Experimental trees of each species were selected and labelled. Details of the selected trees are presented in Table 5. Between 5 and 20 immature spikes were labelled and the following observations and measurements taken: period of anthesis of each spike; length of spike; number of flowers per spike; number of mature pods per spike; period of pod development; and number of seeds per pod.

Table 2. Mean daily climate data at Atherton during the phenology studies.

Year	Month	Mean maximum temp. (°C)	Mean minimum temp. (°C)	Mean rainfall (mm)	Mean relative humidity (%) at 0900 h
1989	February	27.4	18.6	6.5	76.3
	March	25.5	18.1	11.9	87.0
	April	24.6	17.3	7.4	80.0
	May	23.2	15.5	4.1	85.6
	June	21.1	11.6	1.8	81.6
	July	21.0	13.6	0.5	80.0
	August	22.4	11.8	0.5	74.9
	September	27.5	13.2	0.3	59.1
	October	29.4	14.8	0.2	61.7
	November	28.1	17.3	7.8	83.1
	December	28.0	17.3	4.1	79.3
1990	January	28.7	18.3	2.2	82.7
	February	31.0	18.4	1.3	73.0
	March	27.2	18.9	20.4	84.9
	April	25.6	17.0	6.1	85.7
	May	22.9	16.4	5.3	95.0
	June	21.3	12.4	2.5	79.9
	July	21.9	12.0	0.32	80.6
	August	24.5	9.9	0	62.8
	September	22.9	11.9	1.21	72.5
	October	27.1	14.3	0.99	66.1
	November	29.8	16.2	0	69.4
	December	29.7	19.4	9.5	82.4
1991	January	28.2	20.0	21.1	90.7

Table 3. Mean daily climate data at Tawau during the phenology studies.

Year	Month	Mean maximum temp. (°C)	Mean minimum temp. (°C)	Mean rainfall (mm)	Mean relative humidity (%) at 0900 h
1987	March	31.0	21.7	6.0	91.2
	April	31.6	22.7	6.4	89.9
	May	30.6	22.5	6.1	90.9
	June	30.4	22.6	7.1	92.0
	July	30.2	22.0	3.8	93.1
	August	30.3	22.2	4.9	92.5
	September	31.0	21.8	3.0	91.9
	October	31.5	22.3	4.9	91.4
	November	31.3	22.5	7.3	92.5
	December	31.1	22.3	4.9	90.8
1988	January	30.8	21.9	3.6	93.8
	February	30.8	21.9	3.5	90.4
	March	31.2	22.5	9.9	91.5
	April	31.8	22.3	4.5	91.9
	May	31.5	22.6	5.1	92.8
	June	31.3	22.0	4.8	92.1
	July	30.2	21.8	6.8	93.2
	August	30.4	21.7	11.2	92.7
	September	30.5	22.1	7.5	92.4
	October	30.7	22.2	7.6	91.0
	November	30.1	22.3	9.4	89.5
	December	29.9	21.4	3.6	90.7
1989	January	30.5	21.3	9.5	90.5
	February	31.3	21.1	23.8	90.7

Results

Phenology studies

The peak flowering period for both species at Atherton was between March and May, with mature pods present between October and April (Fig. 1, Tables 6 and 7). Flowering was not continuous, however, and flowering and non-flowering periods occurred in cycles, such that the flowering periods of the two species were rarely synchronised. Flowering was much more variable in Sabah, but at both Sepilok and Tawau the main flowering period for *A. mangium* was between December and March (peak January) resulting in mature pods between April and August (peak June–July). There was a second flowering between June and September (peak July–August) but this did not always set fruit, and the yield (August–December) was generally lower than in June–July (Figs 2, 3; Table 6). Flowering and fruiting of *A. auriculiformis* was more sporadic, with a major peak of flowering between May and September (peak July–August) followed by fruiting between November and January (peak December). A second peak of flowering sometimes occurred in March followed by pods in April–May. Additional sporadic peaks of flowering were also observed at other times of year (Figs 2, 3, Table 7). Flowering and fruiting of *A. auriculiformis* at Sepilok was generally between one and two months later than at Tawau.

The phenology assessment categories 1, 2 and 3 corresponded to spike numbers varying between 32 000 and 133 000 for *A. mangium* and between 3 000 and 12 000 for *A. auriculiformis* (Table 8). The correlation coefficients were 0.62 and 0.64 respectively.

Detailed flowering studies

There were large differences in spike length and number of flowers per spike for both species between the different observation sites, despite low variability between trees within each site (Table 9). The period of anthesis of an individual spike was consistently low at between 1.2 and 2.5 days. Most pod shed occurred within one month of anthesis during which period the majority of the spikes were abscissed. No mature pods developed from the spikes at Atherton, and the highest set of 36% of spikes was recorded for *A. mangium* at Tawau in 1988.

Table 4. Mean daily climate data at Sepilok during the phenology studies.

Year	Month	Mean maximum temp. (°C)	Mean minimum temp. (°C)	Mean rainfall (mm)	Mean relative humidity (%) at 0900 h
1987	May	37.4	25.9	2.2	74.0
	June	36.8	26.3	3.2	76.5
	July	36.1	25.7	3.2	79.2
	August	35.7	25.9	7.5	78.8
	September	35.9	25.7	8.0	77.4
	October	35.8	25.9	8.2	78.6
	November	34.7	26.0	8.8	81.8
	December	33.6	25.7	19.3	84.3
1988	January	33.4	25.5	10.7	86.4
	February	33.5	25.7	7.2	85.7
	March	34.5	26.0	3.5	79.4
	April	36.3	26.2	2.2	78.1
	May	36.0	26.3	5.6	76.8
	June	35.7	25.9	11.0	79.5
	July	34.7	25.8	14.0	78.8
	August	35.8	25.6	3.2	78.7
	September	35.8	25.9	7.9	78.4
	October	34.0	25.8	8.6	79.9
	November	31.6	25.7	21.1	81.3
	December	30.1	25.0	33.1	87.5
1989	January	32.3	25.5	6.5	85.1
	February	31.4	25.1	10.0	81.8
	March	33.7	25.3	8.7	81.1
	April	34.6	25.6	4.6	80.9

Discussion

The trees of *A. mangium* and *A. auriculiformis* observed in Malaysia showed higher fertility than those observed in Australia. This was due to both higher flower numbers, and to longer and more frequent periods of flowering in Malaysia than in Australia. The time of flowering was also altered, with a single major peak between March and May for both species in Australia, compared with major peaks in Malaysia in January and July–August for *A. mangium* and *A. auriculiformis* respectively. Australia and Malaysia are in different hemispheres, so a six-month discrepancy in the timing of phenological events may be expected. In fact, there is a three-month delay in the major flowering peak of *A. auriculiformis*, and a three- month advance for that of *A. mangium*. The situation is confounded, however, by the multiple flowering peaks, and the generally sporadic nature of the flowering in Sabah. In addition to the difference in latitude, there are also differences in longitude and climate between the sites. Temperatures in Sabah were generally higher than in Atherton, and there was less variation throughout the year in temperature, rainfall and relative humidity. It should be noted, however, that 1990 was an atypical year in Atherton, with a delayed wet season. Nevertheless, it is possible that the more uniform climate of Malaysia may provide suitable conditions for flowering over a longer period of the year than in Australia. The strong effect of climate on the phenology of the trees is further demonstrated by the fact that a difference in flowering time was detected between Sandakan and Tawau, both in Sabah. The minor flowering period between June and August, and the two periods of pod production for *A. mangium*, were also reported by Gan and Sim (1987). The phenology of *A. mangium* in north-eastern Australia was studied by Hopkins and Graham (1989) over a period of four years. Flowering occurred between January and May, although flower buds were observed as early as November. Pods were present on the trees between May and October, but not all trees produced pod crops in all years (M. S. Hopkins, pers. comm.). Variability in flowering and fruiting was also observed in both species in Thailand (Wasuwanich 1989). *A. auriculiformis* showed generally poor flowering and seed production, whereas *A. mangium* produced more flowers on wet sites.

Mature pod set at Tawau in 1989 was much lower than in 1988, and this may reflect year-to-year variation.

Table 5. Experimental trees used in the detailed flowering studies.

Location	Number of trees observed	Origin of seed	Age of trees at start of observations (years)	Spacing (m)	Seed harvested
Acacia mangium					
Atherton	3	Cape York	5–12	15–80	No
Tawau 92E	5	Ulu Kukut via SAFODA	9	10	Yes
Mandahan	5	Ulu Kukut	5	3	No
Acacia auriculiformis					
Atherton	3	Cape York	5–6	3.5–10	No
Bangawan	5	Ulu Kukut	5	3	No

Table 6. Peaks of flowering and fruiting of *Acacia mangium* in Australia and Malaysia.

Location	Peak no.		Year			
			1987	1988	1989	1990
Atherton	Flowers	1			March	March
	Pods	1			November	November
Tawau	Flowers	1		January		
7432	Pods	1	May	July		
Tawau	Flowers	1		January		
69A		2	July	August		
	Pods	1	June	July		
		2				
Tawau	Flowers	1		January		
143G1		2	August	July		
	Pods	1	May	August		
		2		September		
Tawau	Flowers	1		January		
92D		2	July	July		
	Pods	1	June	April		
		2		September		
Tawau	Flowers	1		January		
35F1		2	August	June		
	Pods	1	June	May		
		2	October	August		
Sepilok	Flowers	1	March	December		
		2		September		
	Pods	1	June	July	June	
		2		December		

1 First flowering peak. 2 Second flowering peak.

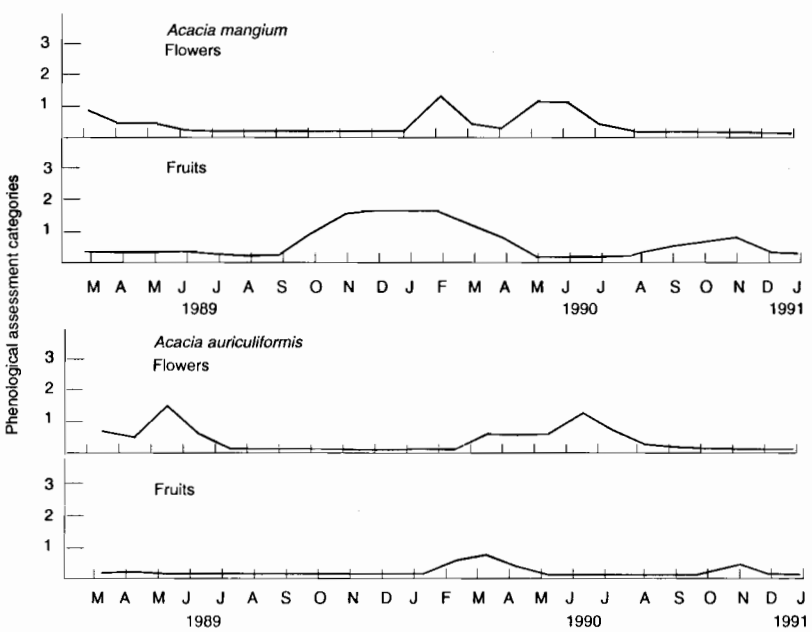

Fig. 1. Flowering and fruiting phenology of *Acacia mangium* and *A. auriculiformis* at Atherton. Phenological assessment categories 1 = light, 2 = medium, 3 = heavy.

Table 7. Peaks of flowering and fruiting of *Acacia auriculiformis* in Australia and Malaysia.

Location	Peak no.		Year			
			1987	1988	1989	1990
Atherton	Flowers	1			May	June
	Pods	1				March
Tawau	Flowers	1				
Arboretum		2	August	June		
	Pods	1	April	April		
		2	December	September		
Tawau	Flowers	1	June			
106G		2	July	September		
	Pods	1	May	November		
		2	December			
Tawau	Flowers	1		July		
24H		2	August			
	Pods	1	March	November		
		2	December			
Tawau	Flowers	1		January		
13F		2	September	August		
	Pods	1	May			
		2		December		
Tawau	Flowers	1		February		
85E		2	August	May		
		3		October		
	Pods	1	May	July		
		2	December	December		
Sepilok	Flowers	1		March	March	
		2	September	August		
	Pods	1	May	May	January	
		2	December		May	

1 First flowering peak
2 Second flowering peak.

Alternatively the difference may be due to experimental procedure, as the spikes in 1988 were labelled 6 days prior to anthesis whereas those in 1989 were labelled 5 weeks prior to anthesis. Of these latter spikes, 45.6% were shed before reaching anthesis, and it is possible that the labelling of such young spikes is detrimental to their subsequent development.

Table 8. Correlations between assessment categories and number of spikes per tree for the phenology studies at Sepilok and Karamunting.

Assessment category	Number of spikes per tree	Correlation coefficient R^2
Acacia mangium		
1	31 971	
2	82 320	0.62
3	132 668	
Acacia auriculiformis		
1	2 603	
2	7 252	0.64
3	11 901	

The assignment of the assessment categories was dependent upon the experience of the observers, and was related to the usual behaviour of the trees in that particular location. Thus the assessments varied between the species with tree age, and between the same species in different locations. Similar patterns were recorded in different populations in similar areas, and this indicates that regular visual assessment is a valid method for the determination of flowering phenology. However, the spike numbers recorded at Sepilok and Karamunting cannot be extrapolated for use in assessments at other locations on trees of different ages. Such detailed information, if required, must be determined at each site.

This study has also highlighted some interesting variation in floral characteristics of the two species. The trees observed in Atherton were consistent in both spike length and flower number per spike. Spikes of *A. mangium*

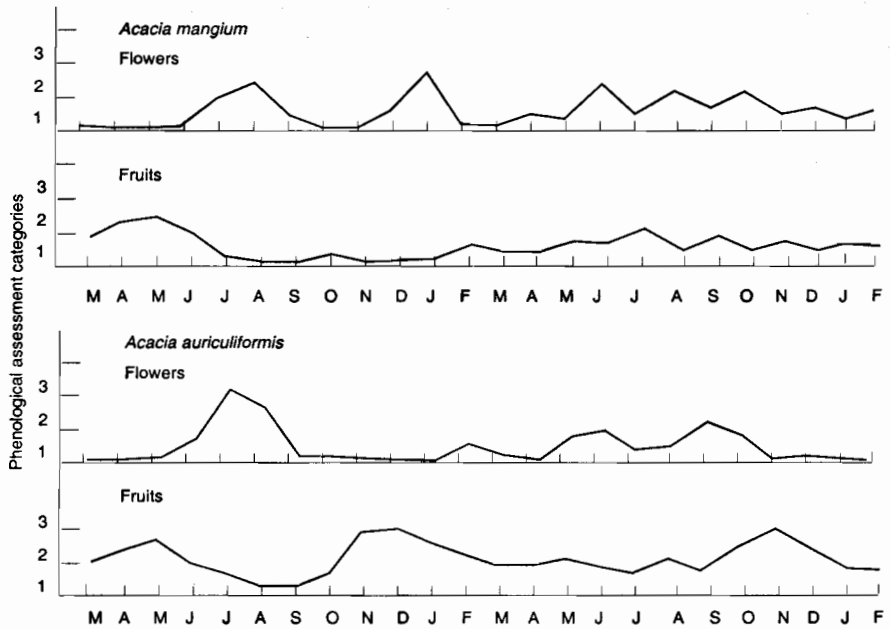

Fig. 2. Flowering and fruiting phenology of *Acacia mangium* and *A. auriculiformis* at Tawau (143G1 and 106G respectively). Phenological assessment categories 1 = light, 2 = medium, 3 = heavy.

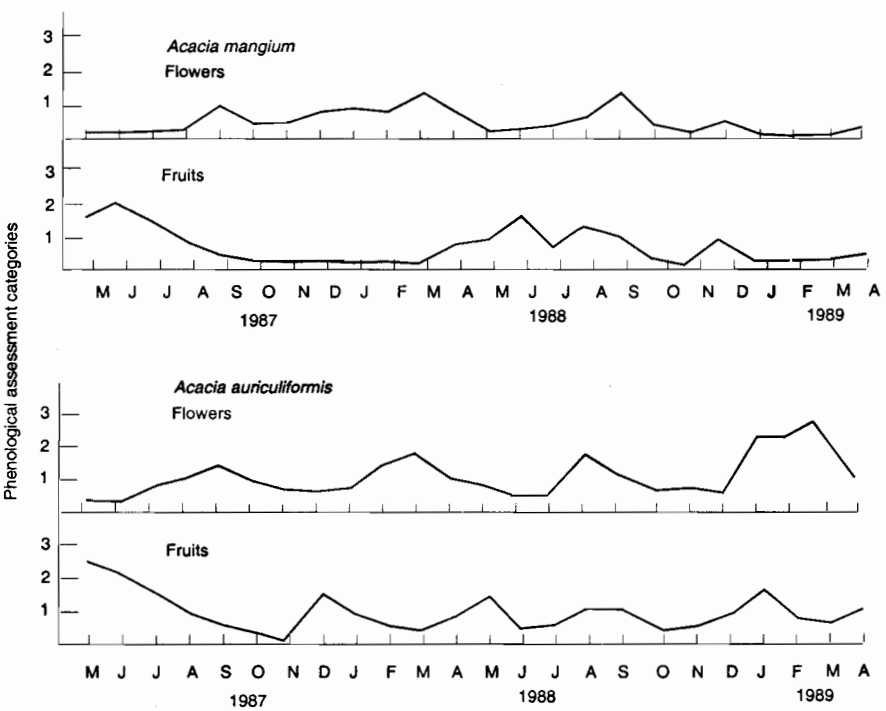

Fig. 3. Flowering and fruiting phenology of *Acacia mangium* and *A. auriculiformis* at Sepilok and Karamunting respectively. Phenological assessment categories 1 = light, 2 = medium, 3 = heavy.

were 9.6 cm in length and had 215.5 flowers, whereas those of *A. auriculiformis* were 5.7 cm with 47.2 flowers. In contrast, the trees observed in Sabah, both in this and previous studies, differed from the Australian trees and were highly variable from site to site (Table 10). It is possible that both species show high variability in spike length and flower number, as all the Australian plants were derived from the Cape York populations, and the taxonomic descriptions for both species cite 8 cm as the spike length (Pedley 1975). Alternatively, it is possible

Table 9. Detailed flowering data for *Acacia mangium* and *A. auriculiformis* in Australia and Malaysia (mean ± standard error).

	Acacia mangium			*Acacia auriculiformis*		
	Atherton 1989	Tawau 1988	Tawau 1989	Mandahan 1987	Atherton 1989	Bangawan 1987
Number of trees	3	5	5	5	3	5
Number of spikes per tree	20	5	46	20	20	20
Spike length (cm)	9.6 ±0.3	9.1 ±0.2		4.1 ±0.8	5.7 ±0.2	5.7 ±0.8
Number of flowers per spike	215.5 ±5.9			209.4 ±4.0	47.2 ±2.1	134.3 ±3.3
Period of spike anthesis (days)	2.5 ±0.3	1.2 ±0.1			1.6 ±0.1	
Days to pod set (2 mm length)			18.5 ±0.4			
Number of set pods per spike			4.0 ±0.3			
Per cent spikes setting pods			21.0			
Per cent pod shed						
<1 month			87.9			
1–2 month			3.7			
2–3 month			2.8			
3–4 month			3.7			
4–5 month			0.5			
5–6 month			0.5			
6–7 month			0.9			
Days to pod maturity		172.6 ±7.6	205.4 ±5.2			
Number of mature pods per spike		1.8 ±0.6	2.8 ±0.3		0	
Number of seeds per pod			5.5 ±0.4			7.7 ±0.5
Per cent flowers maturing pods	0	0.9	0		0	
Per cent spikes maturing pods	0	36.0	5.7	0	0	7.6 ±1.4

Table 10. Comparative flower data for *Acacia mangium* and *A. auriculiformis* (mean ± standard error).

	Spike length (cm)	Number of flowers per spike
Acacia mangium		
Pedley (1975)	up to 8	
Bowen (1981)	7.4	392.2
This study:		
Atherton	9.6 ± 0.3	215.5 ± 5.9
Tawau	9.1 ± 0.2	
Mandahan	4.1 ± 0.8	209.4 ± 4.0
Acacia auriculiformis		
Pedley (1975)	up to 8	
Bowen (1981)	7.2	230.4
This study:		
Atherton	5.7 ± 0.2	47.2 ± 2.1
Bangawan	5.7 ± 0.8	134.3 ± 3.3

that the trees observed in Sabah had already undergone hybridisation and introgression, and that the variable results represent the hybrid nature of the populations. Natural hybridisation between *A. mangium* and *A. auriculiformis* appears to have arisen spontaneously in Sabah, and 'atypical' *A. mangium* trees, which may have hybridised with other *Acacia* species, have also been reported (Gan and Sim 1987). Further clarification of this point is warranted, as these characteristics may have taxonomic significance, and spike characteristics have been quoted as indicative of hybrid status (Bowen 1981). A further study is required of different populations from the range of both species allied to taxonom-ic determination for each accession.

The Australian species *A. mangium* and *A. auriculiformis* both show increased fertility when grown outside of their natural range of distribution in a climate showing reduced yearly variability in temperature, rainfall and relative humidity. It is not unusual for an exotic species to show improved performance over that observed in its native habitat. The vegetative performances of *Pinus radiata* in Australia and New Zealand, and of many species of *Eucalyptus* in various countries around the world, are good illustrations of this phenomenon. Natural interspecific hybridisation between the two species appears to have occurred under these conditions of increased flowering and high fertility, and the superior performance of the hybrid has stimulated the requirement for controlled and directed hybridisation to combine the characteristics of superior parents (Bowen 1981). From the observations made in this study, it appears that the optimum period for hybridisation using *A. mangium* as the female parent is between December and March, and between May and September using *A. auriculiformis* as the female parent. These are the flowering periods which have the highest probability of setting a seed crop in Sabah.

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Flowering and Fruiting Phenology of *Acacia mangium* and *Acacia auriculiformis* in Peninsular Malaysia

Zakaria Ibrahim* and Kamis Awang†

REPORT of the occurrence of natural hybrids between *A. mangium* Willd. and *A. auriculiformis* A. Cunn. ex. Benth. in Sabah (Tham 1976), and the apparent hybrid vigour, in terms of branching habits, observed in the hybrids (Bowen 1981; Rufelds and Jaffirin 1986), have created general interest in planting hybrids in Malaysia.

In initiating a hybridiszation program, one needs to understand the reproductive biology of the species concerned. This covers a wide field such as the floral biology, flowering and fruiting phenology and the breeding system. To a certain extent, the floral biology of *A. mangium* and *A. auriculiformis* has been described by Zakaria and Kamis (1991).

This paper presents the results of a study of the flowering and fruiting phenology of *A. mangium* and *A. auriculiformis* growing in Peninsular Malaysia.

Materials and Methods

Regular observations were made on the flowering and fruiting behaviour of *A. mangium* and *A. auriculiformis* for a period of 23 months (October 1987 to September 1989). For each species, 60 trees were randomly selected and marked. The *A. mangium* trees were planted in 1986 located within the Compensatory Forest Plantation Project area in the Rantau Panjang Forest Reserve. The *A. auriculiformis* trees were planted in 1979 and formed an avenue of trees within a residential area in the vicinity of Forest Research Institute Malaysia (FRIM).

The flowering and fruiting phenology of individual trees were observed once every fortnight, using a pair of 10 × 40 Leitz binoculars. Trees having open flowers were scored as flowering. The flowering intensity was quantitatively designated into four categories, namely: nil (0); light (1) with up to 1/3 of crown flowering; moderate (2) with up to 2/3 of crown flowering; and heavy (3) with more than 2/3 of crown flowering. In determining fruiting intensity, trees with matured pods were considered as fruiting. Fruiting intensity was scored similarly to flowering intensity.

To obtain the monthly flowering and fruiting intensity, the scores of the 60 trees per species, for a particular month, were summed and divided by 180 [60 trees × 3 (highest intensity)] and expressed as percentage of total flowering/fruiting intensity.

Data on the flowering and fruiting of *A. mangium* and *A. auriculiformis* were correlated with climatic factors. Meteorological data (Table 1) on mean monthly temperature and total monthly rainfall were obtained from the Meteorological Station at FRIM.

Results and Discussion

Flowering phenology

In general, both *A. mangium* and *A. auriculiformis* flowered throughout the year with peak flowering periods in certain months of the year. Flowering of both species usually lasted for about a month. The extended period of flowering was due to the progressive development of inflorescences along the twigs and consequent disparity in flowering times. Within trees, anthesis was synchronous. Between trees of a population, anthesis was staggered because of differences in development of inflorescences.

In both species, flowering peaks varied in intensity. For *A. mangium*, the flowering intensity was lower than that of *A. auriculiformis*. The highest flowering intensity in *A. mangium* was 53.8% in July 1989 (Table 2). However, this intensity only fell within the moderate flowering class (33.3 to 66.6% intensity). Moderate flowering was also observed in October 1987. The lowest flowering intensity in *A. mangium* was 1.6 in October, 1988.

For *A. auriculiformis*, heavy flowering was observed in both June 1988 and 1989 in which the intensities exceeded the 66.6% level. The highest flowering intensity was observed in June 1988 (85.6%), while the lowest was 0.6% in January 1989.

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Table 1. Mean monthly temperature and rainfall at FRIM.

Month	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Total rainfall (mm)
1987			
October	32.6	23.0	597
November	31.9	23.3	255.1
December	31.0	22.6	328.1
1988			
January	32.9	22.5	86.4
February	33.2	22.4	376.4
March	33.1	23.2	347.1
April	33.6	23.2	228.6
May	33.5	23.6	192.4
June	33.2	22.8	285.4
July	32.2	22.8	285.4
August	32.0	22.4	297.5
September	32.0	22.6	395.5
October	32.2	22.7	181.7
November	31.0	22.7	332.1
December	31.5	21.3	135.5
1989			
January	32.5	21.9	64.1
February	32.7	21.1	144.5
March	32.3	21.1	245.8
April	32.6	22.7	360.1
May	33.0	23.1	106.6
June	32.9	22.3	131.2
July	32.8	22.2	50.8
August	32.4	22.8	188.6
September	31.9	21.8	365.7

Source: Malaysian Meteorological Service (1987–1989).

Table 2. Monthly intensity of flowering of *A. mangium* and *A. auriculiformis*.

Month	*A. mangium*	*A. auriculiformis*
1987		
October	45.6	26.1
November	24.4	21.6
December	15.5	39.4
1988		
January	2.8	65.6
February	17.8	24.4
March	31.6	46.1
April	27.2	33.8
May	3.8	42.8
June	40.6	85.6
July	32.8	61.1
August	9.4	21.1
September	8.3	36.6
October	1.6	27.8
November	13.3	26.6
December	31.6	33.3
1989		
January	2.2	0.6
March	11.6	3.3
April	12.8	3.3
May	11.6	42.2
June	5.6	74.4
July	53.8	64.4
August	31.1	35.0
September	10.0	10.0

Note: Value expressed as percentage of mean monthly observation; <33.3%, little flowering; 33.3 to 66.6%, moderate flowering; >66.6%, heavy flowering.

The results also indicated that flowering peaks of *A. mangium* and *A. auriculiformis* generally coincided with each other, except in January 1988. During this period, *A. auriculiformis* flowered heavily at 85.6% intensity, while *A. mangium* displayed only 2.8% intensity.

The flowering pattern did not show a satisfactory correlation with mean monthly maximum temperature and monthly rainfall (Table 3), being weak with temperature and very weak with rainfall.

The monthly flowering data of individual trees (n = 60) of both *A. mangium* and *A. auriculiformis* indicated that there were always trees in flower during any month.

Fruiting phenology

As in the flowering, *A. mangium* and *A. auriculiformis* bore fruits throughout the year with some peak fruiting periods. For *A. mangium*, the highest fruiting density was 61.6% in September 1988 and its lowest was 2.2% in April 1989 (Table 4). As for *A. auriculiformis*, heavy flowering (>66.6% intensity) was observed from October to December 1987; April 1988, September to October 1988; and August to September 1989. Its lowest fruiting period was in April 1989 with 0.6% intensity.

The monthly fruiting data of individual trees of both *A. mangium* and *A. auriculiformis* (n = 60) similarly showed that there were trees fruiting at any particular month.

Figures 1 and 2 represent the flowering and fruiting intensities of *A. mangium* and *A. auriculiformis*. The graphs suggest that, for both *A. mangium* and *A. auri-*

Table 3. Regression analysis between flowering intensity of *A. mangium* and *A. auriculiformis* and mean monthly temperature and rainfall.

Regression	Correlation coefficient
A. mangium	
Flowering intensity/temperature	0.1182
Flowering intensity/rainfall	–0.0925
A. auriculiformis	
Flowering intensity/temperature	0.2886
Flowering intensity/rainfall	0.0620

Table 4. Monthly intensity of fruiting of *A. mangium* and *A. auriculiformis*.

Month	*A. mangium*	*A. auriculiformis*
1987		
October	38.3	73.8
November	18.3	75.0
December	9.4	78.3
1988		
January	44.4	39.4
February	35.5	35.0
March	16.6	50.5
April	5.0	76.6
May	23.3	37.2
June	33.8	52.8
July	29.4	41.6
August	10.0	50.6
September	61.1	91.6
October	32.8	71.1
November	14.4	31.1
December	6.6	47.7
1989		
January	2.8	35.0
February	15.0	33.3
March	35.0	30.0
April	2.2	0.6
May	15.0	4.4
June	10.5	6.1
July	12.8	5.6
August	9.4	82.2
September	5.6	85.6

Note: Value expressed as percentage of mean monthly observation; <33.3%, little flowering; 33.3 to 66.6%, moderate flowering; >66.6%, heavy flowering.

culiformis, fruit maturity occurred from three to four months after flowering. The fruiting peaks were distinctly observed after three to four months of peak flowering elapsed.

Conclusions

From the observations made in this study on the flowering phenology, it can be concluded that *A. mangium* and *A. auriculiformis* grown in Peninsular Malaysia flower throughout the year with a distinct flowering season occurring between June and July. Reports from Sabah (Wong, pers. comm.) indicate that the flowering in *A. mangium* there is variable with two peaks in January and July, while flowering in *A. auriculiformis* occurs from July to August and in December. In Papua New Guinea, both *A. mangium* and *A. auriculiformis* flower in April and July (Skelton 1980; Turnbull et al. 1983). In Taiwan, *A. mangium* flowers from October to November and *A. auriculiformis* from July to November (Kiang et al. 1989).

The individual tree phenology suggests that, during the peak flowering, there is flowering synchrony within

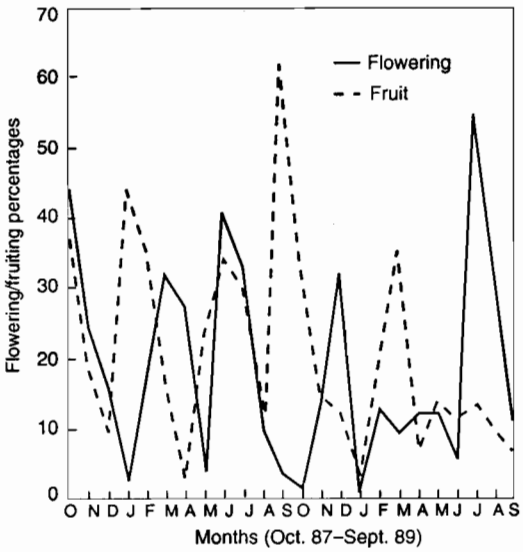

Fig. 1. Monthly flowering and fruiting intensity of *Acacia mangium*.

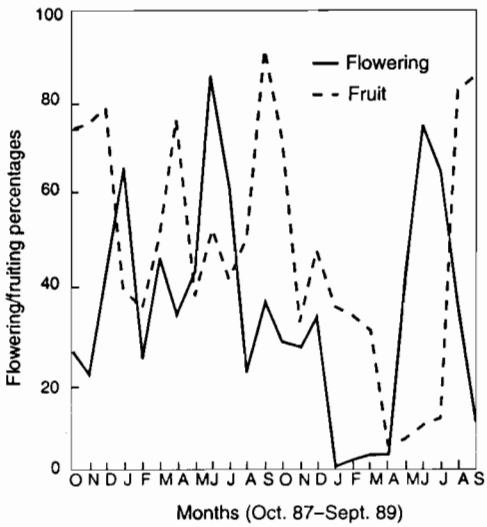

Fig. 2. Monthly flowering and fruiting intensity of *Acacia auriculiformis*.

trees and overlapping of flowering period between *A. mangium* and *A. auriculiformis*. Also between trees, within the same species, there is a staggering of flowering. These phenomena are important in ensuring outcrossing taking place within species and the possibility of occurrence of putative hybrids. Bowen (1981) suggested that the occurrence of natural hybrids between *A. mangium* and *A. auriculiformis* was probably due to the existence of overlapping flowering periods between the two species.

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Preliminary Observations on the Flowering Phenology and Seed Production in a Seedling Hybridising Orchard of *Acacia mangium* and *Acacia auriculiformis*

James Josue*

GENETIC improvement of fast growing tropical trees has gained much attention in the last few decades. Two tropical acacias, *A. mangium* and *A. auriculiformis*, have been widely used for industrial plantations and social forestry programs in Asia. In Indonesia, Malaysia and the Philippines, *A. mangium* is widely planted while in Thailand, India and China, *A. auriculiformis* is of much importance (Turnbull and Crompton 1990).

The discovery of vigorous natural hybrids of *A. mangium* and *A. auriculiformis* in Malaysia has triggered interest in combining the desirable properties of the two species through manipulation. In one of the earliest plantings of *A. mangium* in 1965 at Ulu Kukut, Sabah, *A. auriculiformis* was grown adjacent to *A. mangium*. Spontaneous hybrids produced in this plantation tended to combine the desirable properties of the two species. They often grow more vigorously and have better stem form than *A. auriculiformis* and have lighter branches than *A. mangium*.

Mass propagation techniques being developed to raise large numbers of selected hybrids by vegetative means will allow immediate capture of all the gains in selected clones. Many countries should benefit from the new technique as both species are being used increasingly in Asia and Africa (Turnbull and Crompton 1990).

Owing to an increase in interest in *A. auriculiformis* × *A. mangium* hybrid for reforestation in Sabah, it is desirable that appropriate research projects are initiated to study hybridisation between the two species. Controlled pollination trials have been carried out on marcots from ordinary *Acacia* trees at the Forest Research Centre nursery for many years. There has been little progress towards a better understanding of the ability to successfully control pollinate *Acacia* spp. As the clones used for these trials have not been from selected trees, the resulting seed is not of high genetic quality.

The experimental hybridisation plot described here was thus established at the Gum Gum Forest Reserve. The plot was first proposed in early 1987 and established in June 1987.

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Material and Method

Plot establishment

The plot was set up with an intermixture of *A. mangium* and *A. auriculiformis* at Gum Gum Forest Plantation Station (latitude 5° 51' N, longitude 117° 65' E). Seven *A. auriculiformis* parent trees from Morehead River Provenance, Papua New Guinea (latitude 8° 2' S, longitude 143° 40' E, altitude 70 m) and five *A. mangium* parent trees from Claudie River Provenance, Australia (latitude 12° 44' S, longitude 143°13' E, altitude 60 m) were used in this 0.49 ha plot.

A total of 62 trees was planted in an alternating (by species) arrangement in the plot establishment at a spacing of 10 × 10 metres.

Phenology assessment

For the time being, a phenological study of each tree in the plot, mainly on flowering and fruit development, is in progress. Insect species which aid natural pollination of trees were also observed. This fortnightly phenological observation will extend for about two years in a comprehensive survey of *Acacia* phenology across Malaysia.

Analysis

Positive identification of the percentage of true hybrid seed produced as opposed to selfed seed will be possible by growing the seedlings in the nursery and distinguishing between the taxa using the Seedling Morphology Guide developed by the Forest Research Centre, Sabah. Alternatively, the seed may be differentiated through isozyme studies at the Forestry Research Institute of Malaysia (FRIM), Kepong, Kuala Lumpur.

Preliminary Results and Discussion

At present there is a total of 42 trees in the plot. They are about three years old and have been observed to have started flowering in October 1989. Fortnightly phenological observation on 12 *A. mangium* trees and 14 *A. auriculiformis* trees in the plot started in September, 1990.

The percentage of trees producing floral buds each month is shown in Figure 1. Twenty-five percent of the *A. mangium* trees started to produce buds in November. By that time only 7% of the *A. auriculiformis* had produced buds. In December, all of the *A. mangium* trees were producing buds. The maximum bud production in *A. auriculiformis* was in January (50%).

Figure 2 shows the percentage of trees flowering each month. Flowering of *A. mangium* trees began in January (92%) and was completed by February. The flowering of *A. auriculiformis* trees was somewhat later with only 57% flowering in January.

By March, most of the *A. mangium* trees were fruiting (Fig. 3) but some of the branches of each *A. mangium* tree were still at the flowering stage (Fig. 2). All the *A. auriculiformis* trees were flowering at that time. These species thus have the maximum overlapping flowering period in March, when natural hybridisation is most likely to occur.

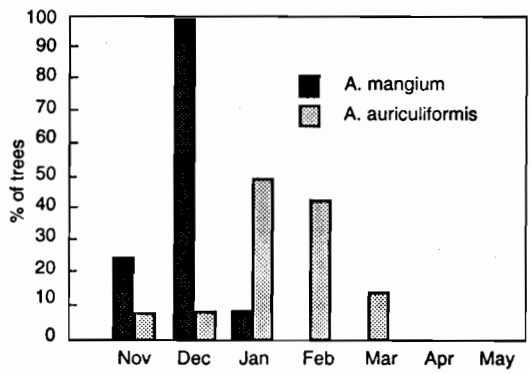

Fig. 1. Percentage of trees producing floral buds.

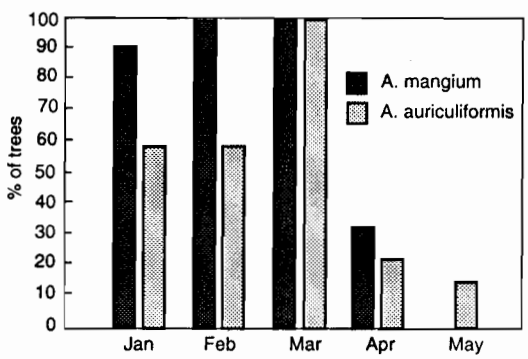

Fig. 2. Percentage of trees flowering.

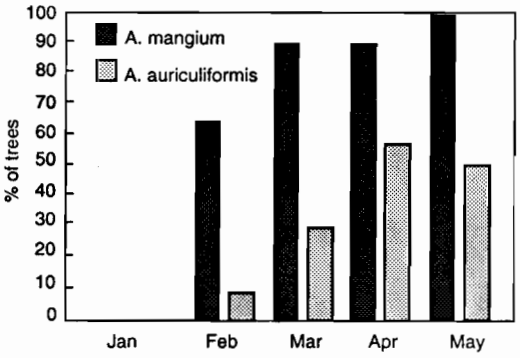

Fig. 3. Percentage of trees fruiting.

The possibility of hybridisation has been noted earlier by Hepburn and Shim and then supported by Pedley who examined some selected herbarium material collected at the Forest Research Centre, Sepilok (Bowen 1981).

In May, all the *A. mangium* were fruiting completely while only 50% of the *A. auriculiformis* were fruiting (Fig. 3). Collection of fruits of the trees will be done when they are mature enough for identification of the percentage of true hybrid seed.

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Insect Visitors to Flowering Branches of *Acacia mangium* and *Acacia auriculiformis*

Margaret Sedgley*, Chey Vun Khen†, Rose M. Smith* and Jane Harbard*

Abstract

Insect visitors to flowering branches of *Acacia mangium* and *A. auriculiformis* in Malaysia and Australia were collected and identified. Insects collected in Sabah were examined by scanning electron microscopy for the presence of pollen on their ventral surfaces. A range of insect types was observed on the trees in Australia, but bees were the most common visitors to *Acacia* flowers in Sabah. Numerous polyads adhered to their hairy bodies and the bees collected polyads in their pollen baskets. Most insect visitors had only *Acacia* polyads on their bodies, with the exception of some dipterans which had Asteraceae pollen grains. Most of the insects foraged for pollen, although a minority foraged also for nectar. The insects with hairy bodies had most *Acacia* polyads on their ventral surfaces, suggesting that they may act as pollen vectors. *Trigona* and *Apis* bees, and those belonging to the Colletidae, carried the most pollen.

THIS study was undertaken to gain information on the insects which may be acting as pollinators of *Acacia mangium* and *Acacia auriculiformis*. A knowledge of the insect pollinators of the tropical acacias is particularly important in the context of hybridising orchards. If species are to be interplanted for the routine production of hybrid seed for plantation establishment, then attention must be paid to the provision of efficient pollen vectors to transfer the pollen from tree to tree. Even a low proportion of selfed seed may be detrimental to the quality of the overall plantation established using that seed. A decision must be made on whether to rely on natural insect populations in the hybridising orchard, or to take the expensive step of introducing honeybee hives during the peak flowering period for the purpose of pollination. If the natural pollinators are known, then a less- expensive compromise may be to encourage the populations of effective pollinators by providing additional food sources or nesting sites.

Most acacias which have been studied are outcrossers, and require pollen vectors to transfer the pollen from the anther to the stigma (Sedgley 1986, 1989). Depending on the species, these vectors may be insects (Bernhardt and Walker 1985) or birds (Ford and Forde 1976), with bees reported to be the most common group associated with acacias (Bernhardt and Walker 1984). Pollen vectors are often attracted to the flowering branches to collect nectar from extrafloral glands located on the phyllodes (Sedgley 1986, 1989). Most studies have been conducted on the species native to southern Australia, little attention being paid to the tropical members of the genus.

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Materials and Methods

Studies were conducted at Atherton in northern Australia and at Sepilok, Karamunting and Tawau in Sabah. Trees included the planted specimens described in the phenology and hybridisation studies (Sedgley et al., these proceedings), and those at Atherton were close to the native distribution of the two species. Insects observed visiting the flowering branches of trees of each species were recorded, and specimens of each type caught for later identification. The observations were made during the fortnightly phenology observation periods at Sepilok and Karamunting in 1988, and during the pollination experiments at Atherton in 1989.

More detailed studies were conducted in 1989 and 1990 in Sabah. Collections involved five trees of *A. auri-*

culiformis and *A. mangium* at Karamunting and Sepilok during March and September 1989 respectively. Observations were made on whether the insect visitors foraged for pollen or for nectar. Flowers from one flowering branch on each tree were swept with an insect net every two hours from 0630 to 1600 h for five consecutive days. Insects were also collected from flowering branches of trees of both species at Tawau in 1990. All insects collected were stored individually in gelatin capsules and sent to Adelaide for identification and further observation. Where present, the pollen baskets were removed and dispersed on a microscope slide for counting of the pollen grains. Specimens were prepared for observation by scanning electron microscopy (SEM) by placing the insect on its back on a SEM stub and coating with gold. Each insect was examined for pollen grains on its ventral surface, and the numbers of acacia polyads and foreign pollen grains were recorded.

Results

A range of insect visitors including representatives of the orders Hymenoptera, Coleoptera, Lepidoptera, Hemiptera and Diptera were observed on the flowering branches in Australia (Table 1). Members of the bee genus *Trigona* were the most common visitors to the *A. mangium* trees at Sepilok, with a species of *Phanerotoma* (Braconidae) identified visiting the flowering branches of *A. auriculiformis* at Karamunting (Table 2).

In the SEM studies, the most common visitors to the flowering branches of *A. mangium* at Sepilok were again *Trigona* species. Over the five-day period 16 *Trigona* were collected from the five trees,each with an average of 136 *Acacia* polyads on its hairy body (Table 3, Figs 1, 2, 3). The *Trigona* also had pollen baskets on their legs which contained an average of 3885 polyads per basket. Lower numbers of the other insect types were collected, of which only the *Apis* (honeybee) had large numbers of pollen grains on the body and possessed pollen baskets. All types had only acacia polyads on the body, except for one of the Dipterans on which five Asteraceae pollen grains were observed (Fig. 4). All the insects foraged for pollen, although some *Trigona* and eumenid wasps also foraged for nectar. The most common insects observed visiting the flowering branches of *A. auriculiformis* at Karamunting were beetles, with 13 individuals of *Harmonia sedecimnotata* (Fabricius) collected (Table 4). All groups except Homoptera, Diptera and Psocoptera had polyads on their bodies, but the numbers were very low. This may be due to the fact that many of the insects had few or short hairs on their bodies (Figs 5, 6), although this was not true of the braconid wasps (Figs 7, 8) which also had low numbers of polyads. No pollen grains other than those of *Acacia* were present on any of the insects, and all were observed to forage only for pollen. Native bees carried the most pollen grains of the trees sampled at Tawau in 1990 (Table 5). As in 1989, a Dipteran collecting from *Acacia* flowering branches had Asteraceae pollen grains on its body.

Table 2. Insect visitors collected from flowers of *Acacia mangium* at Sepilok and *Acacia auriculiformis* at Karamunting in 1988.

Acacia mangium

Hymenoptera: Apidae
- *Trigona apicalis* Smith[1]
- *Trigona canifrons* Smith[1]
- *Trigona (Tetragonilla) collina* Smith[1]
- *Trigona binghami* Schwarz (possibly synonymous with *T apicalis*)[2]
- *Trigona fuscibasis* Cockerell (similar to *T collina*)[2]

Acacia auriculiformis

Hymenoptera: Braconidae
- *Phanerotoma* sp.[3]

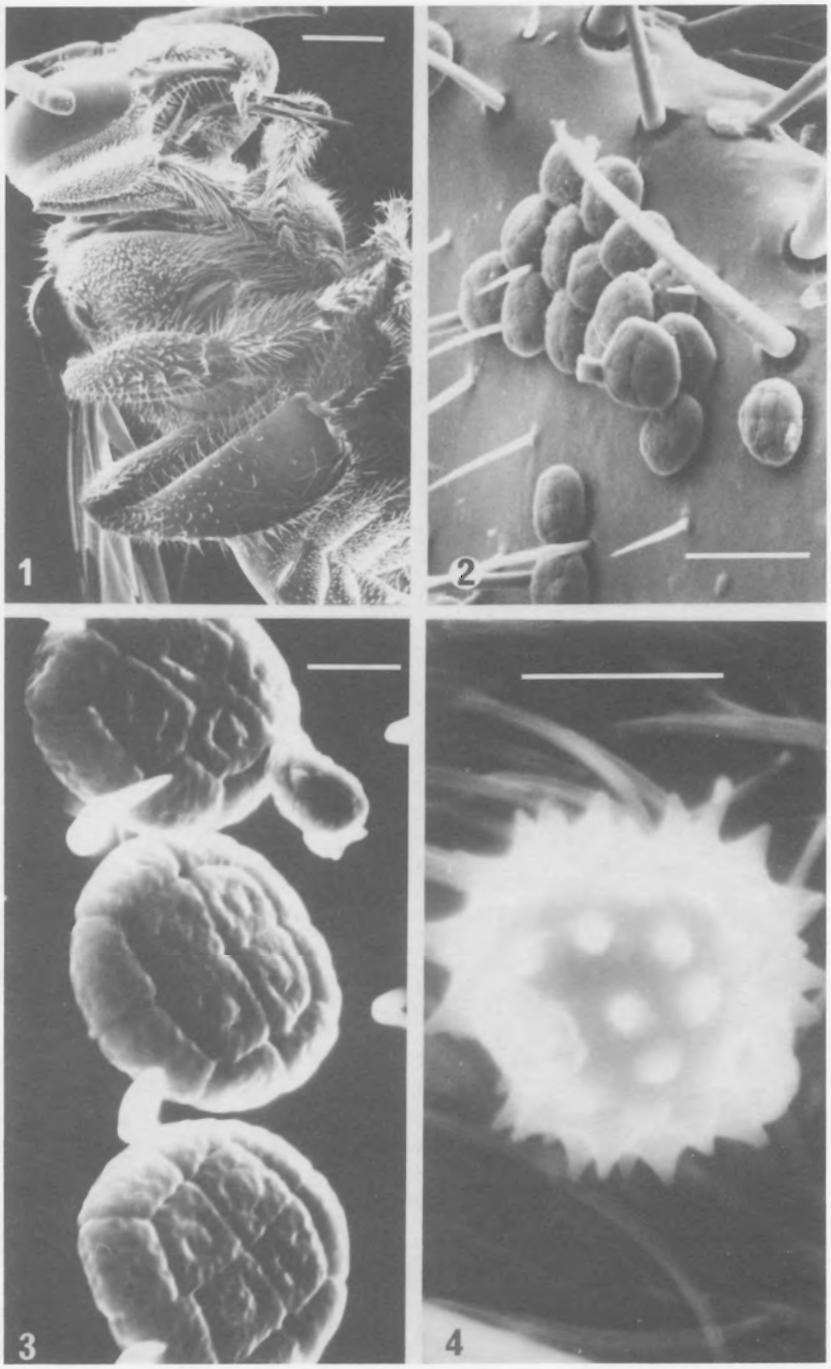
[1] Identified by D.B. Baker, Natural History Museum, London
[2] Identified by S.G. Khoo, University of Malaysia
[3] Identified by A.K. Walker, Commonwealth Institute of Entomology

Discussion

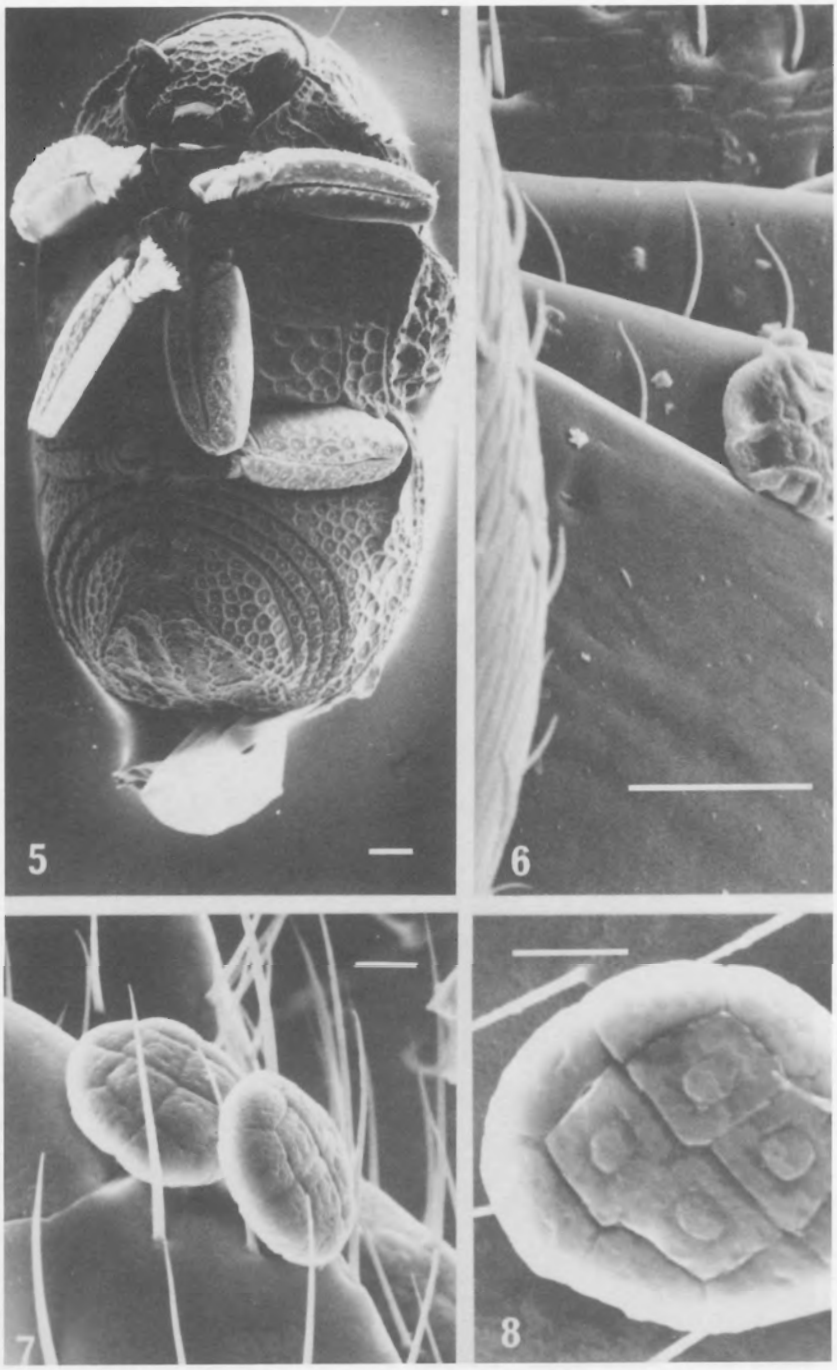
The numbers of insects observed visiting the flowering branches of *A. mangium* and *A. auriculiformis* were low, and a different spectrum of insects was collected from the two species at Sepilok and Karamunting. The two stands of trees were separated geographically by 40 km,

Table 1. Insect visitors collected from flowers of *Acacia mangium* at Atherton in 1989.

Order	Sub-order	Family	Sub-family	Species
Coleoptera		Chrysomelidae	Chrysomelinae	
Lepidoptera	Dytrisia			
Hemiptera		Reduviidae (exuvia)		
Diptera		Syrphidae		
Hymenoptera		Apidae		*Apis mellifera* L.



Figs 1–4. Insects collected from flowering branches of *Acacia mangium* in Sabah. **1.** *Trigona* sp. showing hairy body. Bar represents 500 μm. **2.** Polyads on the leg of a *Trigona* sp. Bar represents 50 μm. **3.** Polyads on the tail of a *Trigona* sp. Bar represents 10 μm. **4.** Asteraceae pollen on the thorax of a dipteran. Bar represents 10 μm.



Figs 5–8. Insects collected from flowering branches of *Acacia auriculiformis* in Sabah. **5.** Coleopteran showing body free from hairs. Bar represents 100 µm. **6.** Polyad on the abdomen of a chrysomelid. Bar represents 10 µm. **7.** Polyads on the leg of *Bracon* sp. Bar represents 10 µm. **8.** Polyad on the wing of *Bracon* sp. Bar represents 10 µm.

and so were visited by different populations of insects. *Trigona* were the most numerous insect visitors to *A. mangium* flowers and had the highest numbers of polyads on their hairy bodies. They were considered to be potentially the most effective of the native pollinators. Although they were not observed to visit the *A. auriculiformis* trees at Karamunting, they have been observed to visit trees of this species in other parts of Sabah, and so could be considered as potential interspecific pollinators. Moreover, native bees belonging to the Colletidae were observed to visit flowering branches of both species at Tawau, and carried large numbers of *Acacia* polyads on their bodies. This is of importance as the production of hybrid seed depends upon active foraging on the flowers of both species. All insect groups visiting both species were observed to forage for pollen,

Table 3. Pollen on bodies of insects collected from flowers of five trees of *Acacia mangium* at Sepilok in September 1989.

Insect type	Number of individuals observed	Mean number of *Acacia* polyads per insect (±SE) body	Mean number of *Acacia* polyads per pollen basket (±SE)	Mean number and type of pollen grains (not *Acacia*)	Collecting pollen (P) or nectar (N)
Hymenoptera					
Apidae					
Trigona sp.	16	136.3±92.2	3885.4±625.9	0	P and N
Apis sp.	1	714.0	6672.0	0	P
Vespidae	3	5.0±2.0	–	0	P
Eumenidae	2	0	–	0	P and N
Diptera	4	1.2±1.2	–	5.0±5.0 Asteraceae	P
Coleoptera	1	0	–	0	P

Table 4. Pollen on bodies of insects collected from flowers of five trees of *Acacia auriculiformis* at Karamunting in March 1989.

Insect type	Number of individuals observed	Mean number of *Acacia* polyads per insect (±SE)	Mean number and type of pollen grains (not *Acacia*)	Collecting pollen (P) or nectar (N)
Coleoptera				
Chrysomelidae: *Coenobius* sp. *Nodina* spp. *Bucharis* sp.	25	1.9±0.9	0	P
Coccinellidae: *Harmonia sedecimnotata* (Fabricius)	13	0.6±0.5	0	P
Others	8	0.4±0.3	0	P
Hymenoptera				
Braconidae				
Bracon sp.:	14	1.9±1.3	0	P
Others	7	0.1±0.1	0	P
Hemiptera				
Homoptera	5	0	0	P
Heteroptera	2	0.5±0.5	0	P
Diptera	3	0	0	P
Psocoptera	1	0	0	P

Table 5. Pollen on bodies of insects collected from flowers of *Acacia mangium* and *Acacia auriculiformis* at Tawau in September 1990.

Insect type	Number of individuals observed	Mean number of *Acacia* polyads per insect	Mean number of *Acacia* polyads per pollen basket	Mean number and type of pollen grains not *Acacia*
Acacia mangium				
Hymenoptera				
Colletidae	1	233	20 882	0
Acacia auriculiformis				
Hymenoptera				
Colletidae	1	800	12 508	0
Diptera				
Muscidae	1	0	–	10 (Asteraceae)
Coleoptera				
Curculionidae	1	3	–	0

and those with pollen baskets had full loads of polyads. Thus all had the potential to act as pollen vectors, although the low occurrence of foreign pollen may indicate a reluctance to fly from one plant to another.

The most successful insect pollinator of plants is *Apis*, the honeybee, due to its acceptance of human-provided hives, its practice of foraging to provide food for its young, and its hairy body to which large numbers of pollen grains adhere. Honeybee hives are introduced at flowering time to improve pollination of a wide range of tree crops (Sedgley and Griffin 1989). This is an expensive practice, however, as the culture of strong, active hives is a specialist occupation which incurs a cost either in the employment of labour or in the hiring of hives. *Trigona* bees are also communal, and preliminary attempts to hive them have shown some success. This may be a cheaper alternative to honeybees, either by culture in honeybee-type hives or by leaving nesting sites, in the form of large hollow dead trees, in or near to the acacia plantation. The use of pesticides in the plantation and neighbouring areas would need to be carefully monitored to avoid the poisoning or repulsion of the native bees.

This work has shown that many of the insect visitors to flowering branches of *A. mangium* and *A. auriculiformis* have the potential to be pollen vectors through their foraging for *Acacia* pollen, and the adherence of polyads to their bodies. Further research is needed on the problem of low numbers of insect visitors to the flowering branches.

Acknowledgments

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Pod Production and Hybrid Seed Yield in *Acacia mangium* and *Acacia auriculiformis*

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Abstract

Spontaneous hybrid seed yield in 10 year old *Acacia mangium* and *A. auriculiformis* planted in adjacent blocks was determined at 3 pod production periods each using isozyme analysis. Temporal variation in hybrid seed yield was significant in both species. On average, hybrid seed yield varied from 0.7 to 21.7% in *A. mangium* and 2.9 to 14.7% in *A. auriculiformis* at the different pod production periods. Average spontaneous hybridisation rate ranged from O to 24.0% in *A. mangium* (mean = 6.9%) and from 2.2 to 25.7% in *A. auriculiformis* (mean = 9.3%). Genotype cross-incompatibilities were observed in *A. mangium* but not in *A. auriculiformis*.

Acacia mangium Willd. and *A. auriculiformis* A. Cunn. ex Benth. are two tropical acacias natural to Australia, Papua New Guinea and Indonesia which have potential for timber and pulp production. Spontaneous hybrids of these two species have been reported in natural populations (Skelton 1987, Gunn et al. 1989) and from plantation grown trees in Sabah (Sim 1987) and Taiwan (Kiang et al. 1989). There is considerable interest in breeding these hybrids which tend to combine the desirable growth characteristics of the parent species (Griffin 1988).

The hybrids tend to grow vigorously, have better form than *A. auriculiformis* and have lighter branching than *A. mangium* which self-prune (Rufelds and Lapongan 1986).

Manipulated hybrids of *A. mangium* and *A. auriculiformis* have been successfully produced by hand pollination (Wickneswari et al.1989, Sedgley et al.of these proceedings). The yield of hybrids depends on the hybridisation technique used. Production of 100% manipulated hybrids has been reported using emasculated flowers (Sedgley et al. of these proceedings) which is a laborious task in acacias. Another option for producing these hybrids is by establishing biclonal hybridising orchards of select *A. mangium* and *A. auriculiformis* trees. Hence, this study was carried out to estimate the spontaneous hybridisation rate between *A. mangium* and *A. auriculiformis* which might be expected in an orchard.

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Materials and Methods

Study site

Acacia mangium and *A. auriculiformis* trees planted in adjacent blocks at Bukit Hari, Forest Research Institute Malaysia, Kepong were chosen for this study. Fig. 1 shows the layout of the *A. mangium* and *A. auriculiformis* stands. There are 50 trees in the *A. mangium* plot and 291 trees in the *A. auriculiformis* plot.

Both these stands were established in 1981 with a planting distance of 3 m × 3 m. Seeds from 11 *A. mangium* trees and 20 *A. auriculiformis* trees, planted in adjacent rows (Fig. 1), were analysed for spontaneous hybrids. The distance between the two most adjacent rows of *A. mangium* and *A. auriculiformis* is 10 m.

Phenological observations and seed collection

Intensity of open flowers, developing pods and mature pods were recorded fortnightly for the 11 *A. mangium* and 20 *A. auriculiformis* trees. *Acacia mangium* produced pods in January 1990, April 1990, and April 1991 whereas *A. auriculiformis* produced pods in October 1989, April 1990 and October 1990. At each pod production period,

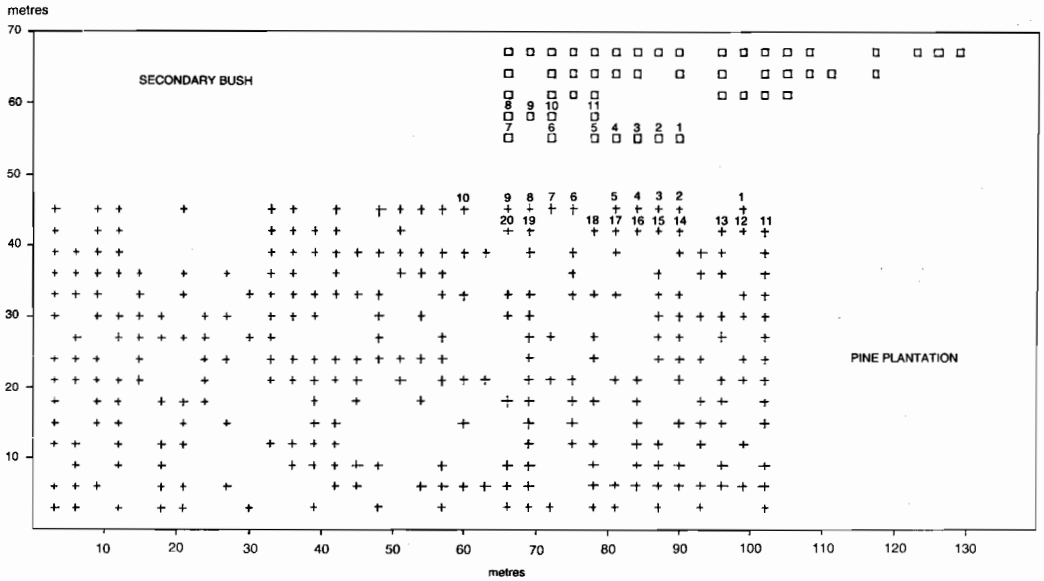

Fig. 1. Plot layout of *A. mangium* and *A. auriculiformis* at Bukit Hari, FRIM. Key: □, *A. mangium*; +, *A. auriculiformis*.

5–10 g of seeds were harvested from each tree. The seeds were extracted from their pods and stored in plastic bags at 10°C until used for isozyme analysis.

Isozyme analysis

Leaf tissues were used to determine the isozyme genotypes of the *A. mangium* and *A. auriculiformis* parent trees. Routinely about 50 open-pollinated seeds from each parent tree were analysed for occurrence of spontaneous hybrid seeds. Details of electrophoretic techniques and staining methods are given by Wickneswari and Norwati (1991).

Each parent tree was scored for electrophoretic variants at 15 loci from 9 enzyme systems viz. aspartate aminotransferase, E.C. 2.6.1.1 (AAT), diaphorase, E.C.1.6.4.3 (DIA), glutamate dehydrogenase, E.C. 1.4.1.2 (GDH), isocitrate dehydrogenase, E.C. 1.1.42 (IDH), malate dehydrogenase, E.C. 1.1.1.37 (MDH), menadione reductase, E.C. 1.6.99.2 (MR), peroxidase, E.C. 1.11.1.7 (PER), phosphoglucomutase, E.C. 2.7.5.1 (PGM) and phosphogluconate dehydrogenase, E.C. 1.1.1.44 (PGD). Each progeny was scored for electrophoretic variants at Aat-1, Gdh-1 and Per-1.

Results

Isozyme genotypes of parent trees

Of the 15 loci scored for, only 5 loci viz Aat-1, Aat-2, Gdh-1, Per-1 and Pgd-1 were variable either within and/or between the *A. mangium* and *A. auriculiformis* parent trees. Dia-1, Idh-1, Mdh-1, Mdh-2, Mr-1, Mr-2, Mr-3, Per-2, Pgm-1 and Pgm-2 were invariant in both parents. Table 1 shows the isozyme genotypes of the parent trees at the variable loci. All the *A. auriculiformis* parents were of the same genotypes at these 5 variable loci. In the case of the *A. mangium* parents, they had the same genotypes at 4 of the 5 variable loci. However,the *A. mangium* parents differed from the *A. auriculiformis* parents at 3 loci viz. Aat-1, Gdh-1, and Per-1, where the alleles present in the *A. mangium* parents were not present in the *A. auriculiformis* parents and vice versa.

Phenological observations

Figure 2 shows the frequency and intensity of flowering in *A. mangium* and *A. auriculiformis* parent trees for the period January 1990 to May 1991. During this period, the *A. mangium* parents produced pods on three occasions viz. January 1990, April 1990, and April 1991 whereas the *A. auriculiformis* parents produced pods twice viz. April 1990 and October 1990. Two periods of synchronous flowering were observed in these two species between January 1990 and May 1991 (Fig. 2). The first overlap in flowering in January–February 1990 led to pod production in both species in April 1990. However, the second overlap in December 1990–January 1991, led to pod production in *A. mangium* only in April 1991. Earlier phenological observations by Zakaria (pers. comm.) in this plot showed an overlap in flowering in June–July 1989. This, however, led to pod production in *A. auriculiformis* only in October 1989 the seed crop of which was analysed in this study.

Table 1. Isozyme genotypes of *Acacia mangium* (Am) and *A. auriculiformis* (Aa) parent trees.

Mother tree[1]	Isozyme genotypes[2] Loci				
	Aat-1	Aat-2	Gdh-1	Per-1	Pgd-1
Am 1	22	11	22	22	12
Am 2	22	11	22	22	12
Am 3	22	11	22	22	12
Am 4	22	12	22	22	12
Am 5	22	12	22	22	12
Am 6	22	12	22	22	12
Am 7	22	12	22	22	12
Am 8	22	12	22	22	12
Am 9	22	12	22	22	12
Am 10	22	12	22	22	12
Am 11	22	11	22	22	12
Aa 1	11	11	11	11	22
Aa 2	11	11	11	11	22
Aa 3	11	11	11	11	22
Aa 4	11	11	11	11	22
Aa 5	11	11	11	11	22
Aa 6	11	11	11	11	22
Aa 7	11	11	11	11	22
Aa 8	11	11	11	11	22
Aa 9	11	11	11	11	22
Aa 10	11	11	11	11	22
Aa 11	11	11	11	11	22
Aa 12	11	11	11	11	22
Aa 13	11	11	11	11	22
Aa 14	11	11	11	11	22
Aa 15	11	11	11	11	22
Aa 16	11	11	11	11	22
Aa 17	11	11	11	11	22
Aa 18	11	11	11	11	22
Aa 19	11	11	11	11	22
Aa 20	11	11	11	11	22

[1] Mother trees are denoted by two alphabets which state the species and a number which shows the position of the tree in the plot (Fig. 1).

[2] Isozyme genotypes are denoted by allelic combinations at a given locus. Alleles are represented by numbers, where 1 is the most anodal, 2 is the next most anodal , etc. The enzymes are as follows: aspartate aminotransferase, AAT; glutamate dehydrogenase, G.D.H; peroxide, PER and phosphogluconate dehydrogenase, PGD.

Besides the peaks in flowering and fruiting noted above, sporadic flowering and fruiting was observed in the *A. mangium* and *A. auriculiformis* plots.

Spontaneous hybridisation

Spontaneous hybrids between *A. mangium* and *A. auriculiformis* were detected by assaying open-pollinated progenies for glutamate dehydrogenase. Hybrids yielded a heterozygous genotype at Gdh-1. Open-pollinated progenies from *A. mangium* mother trees were either of a 22 genotype (selfs) or 12 genotype (hybrids); whereas open-pollinated progenies from *A. auriculiformis* mother trees were either of a 11 genotype (selfs) or 12 genotype (hybrids).

Table 2 summarises the percentage of spontaneous hybridisation in the 11 *A. mangium* and 20 *A. auriculiformis* trees at different pod production periods. Temporal variation in spontaneous hybridisation rate was significant in both *A. mangium* (mean = 0.7 to 21.7%) and *A. auriculiformis* (mean = 2.9 to 14.7%). The spontaneous hybridisation rate also varied significantly between mother trees (mean = 0.0 to 25.7%). The mean spontaneous hybridisation rate for *A. mangium* mother trees was 6.9% whereas for *A. auriculiformis* mother trees it was 9.3%.

Figures 3 and 4 demonstrate the relationship between fruiting intensity and hybrid seed yield in *A. mangium* and *A. auriculiformis* respectively. A decrease in both pod production and hybrid seed yield was observed in both *A. mangium* and *A. auriculiformis* between October 1989 and April 1991.

Discussion

The isozyme locus Gdh-1 was used as the genetic marker to identify hybrids between *A. mangium* and *A. auriculiformis* because earlier studies have shown that

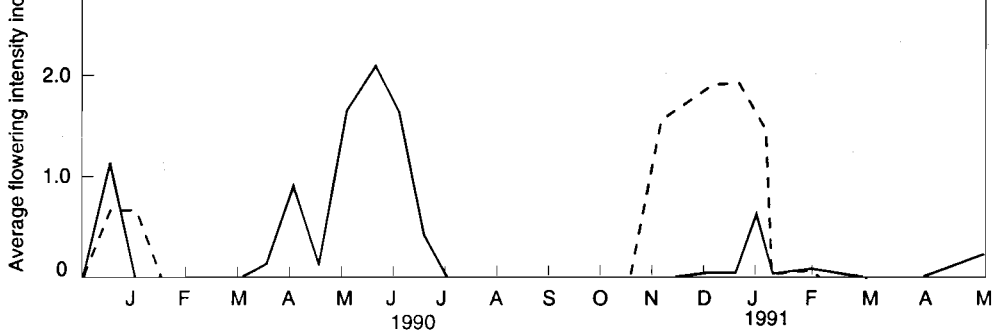

Fig. 2. Synchronous flowering in *Acacia mangium* and *A. auriculiformis* at Bukit Hari. Solid line = *A. mangium*; broken line = *A. auriculiformis*

Table 2. Percentage of spontaneous hybridisation* between *A. mangium* and *A. auriculiformis* at different pod production periods.

Mother tree	Pod production period					
	October 1989	January 1990	April 1990	October 1990	April 1991	Mean per tree
Am 1	–	18.4	0.0	–	0.6	6.3
Am 2	–	5.7	0.0	–	0.0	1.9(*)
Am 3	–	40.0	6.0	–	0.0	15.3**
Am 4	–	32.5	0.0	–	2.0	11.5**
Am 5	–	20.0	4.0	–	0.0	8.0
Am 6	–	0.0	0.0	–	0.0	0.0(*)
Am 7	–	14.3	0.0	–	–	7.2
Am 8	–	60.0	8.0	–	4.0	24.0**
Am 9	–	–	0.0	–	0.0	0.0(*)
Am 10	–	–	0.0	–	0.0	0.0(*)
Am 11	–	4.0	0.0	–	0.0	1.3(*)
Aa 1	26.7	–	22.0	4.0	–	17.6**
Aa 2	30.0	–	0.0	2.0	–	10.7
Aa 3	16.7	–	15.4	2.0	–	11.4
Aa 4	13.3	–	4.0	2.0	–	6.4
Aa 5	16.7	–	4.0	0.0	–	6.9
Aa 6	16.7	–	0.0	6.0	–	7.6
Aa 7	16.7	–	26.0	4.0	–	15.6**
Aa 8	6.6	–	2.0	6.0	–	4.9(*)
Aa 9	6.6	–	0.0	0.0	–	2.2(*)
Aa 10	6.6	–	0.0	0.0	–	2.2(*)
Aa 11	3.3	–	26.0	0.0	–	9.8
Aa 12	10.0	–	10.0	8.0	–	9.3
Aa 13	3.3	–	26.0	0.0	–	9.8
Aa 14	13.3	–	4.0	4.0	–	7.1
Aa 15	3.3	–	0 0	2.0	–	1.8(*)
Aa 16	13.3	–	57.8	6.0	–	25.7**
Aa 17	33.3	–	8.9	8.0	–	16.7**
Aa 18	10.0	–	0.0	0.0	–	3.3(*)
Aa 19	26.7	–	2.0	0.0	–	9.6
Aa 20	20.0	–	0.0	4.0	–	8.0
Mean per Am pod		21.7**	1.6(*)	0.7(*)	6.9	
Production period Aa	14.7**	10.4	2.9(*)	9.3		

* Percentage of spontaneous hybridisation rate is based on 50 progenies per tree at each pod production period with the exception of October 1989 where 30 progenies per tree were assayed.
– No pods were produced.
** Significantly higher than the grand mean value (Am=6.9% ; Aa=9.3%) at p = 0.05
(*) Significantly lower than the grand mean value (Am=6.9% ; Aa=9.3%) at p = 0.05

A. mangium has a 22 genotype at Gdh-1 whereas *A. auriculiformis* has a 11 genotype (Wickneswari 1989, Wickneswari and Norwati 1991). Furthermore, this locus is non-tissue specific (Wickneswari 1989). In this study, besides Gdh-1, Aat-1 and Per-1 were also discriminatory between the two parental species. However, these two loci can only be used to detect hybrids of controlled crosses between these parental species. Since open-pollinated progenies of each parent tree were assayed, outcrosses within the species (isozyme genotypes of all the trees in the plot were not determined) could also have been scored as hybrids as Aat-1 and Per-1 are polymorphic in *A. mangium* (Wickneswari, unpbl.) and *A. auriculiformis* (Moran et al. 1989, Wickneswari and Norwati 1991). In fact, some outcrosses were detected in both species in this study (results not presented here).

Variations in timing and intensity of flowering and fruiting observed in *A. mangium* and *A. auriculiformis*

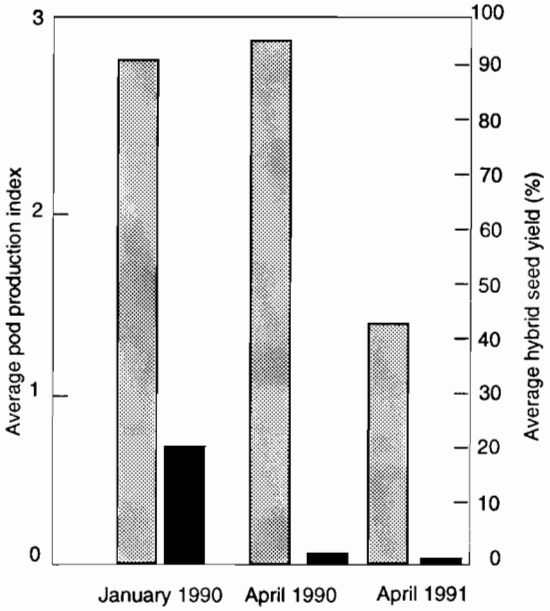

Fig. 3. Pod production and hybrid seed yield in *Acacia mangium*: hatched bar = pod production; solid bar = hybrid seed yield. Average Pod Production Index: 0 – no pod production; 1 – little pod production; 2 – medium pod production; 3 – heavy pod production.

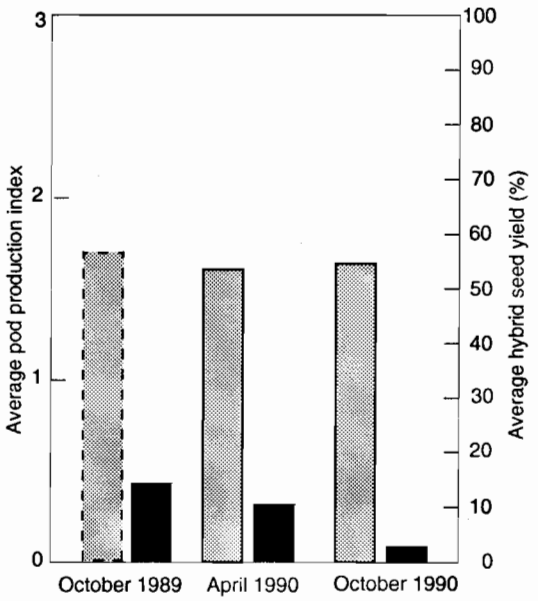

Fig. 4. Pod production and hybrid seed yield in *Acacia auriculiformis:* hatched bar = pod production; solid bar = hybrid seed yield. Average Pod Production Index: 0 – no pod production; 1 – little pod production; 2 – medium pod production; 3 – heavy pod production.

could be due to environmental influences. Sometimes, fruiting did not follow flowering. This was especially so, when the intensity of flowering was low. This phenomenon was observed both in *A. mangium* (flowering in June–July 1989 did not lead to pod production) and *A. auriculiformis* (flowering in December 1990– January 1991 did not lead to pod production).

Even though synchronous flowering was observed in *A. mangium* and *A. auriculiformis* every year, this did not occur at the same time in each of the years. In 1989, synchronous flowering was noted in June–July (Zakaria, pers. comm.); whilst in 1990 it occurred in January–February and December and in 1991 it occurred in January. Hybrid yields in the seed crops resulting from these synchronous flowerings were variable. Generally, a decrease in hybrid yield was observed from 1989 to 1991 in both *A. mangium* (Fig. 3) and *A. auriculiformis* (Fig. 4). This temporal variation in hybrid yield could be due to behavioural changes of the insect pollinators and to environmental influences. The average hybrid seed yield was disappointingly low (< 10%) for an operational biclonal hybridising orchard. However, in a biclonal hybridising orchard, the opportunities for intraspecific outcrossing would be reduced, hence a higher yield of interspecific hybrids would be expected. Regular production of hybrids can also be ensured, and hybrid yields increased, by artificial induction of flowering and culturing insect pollinators in the orchard to ensure good seed set. This possibility requires further research. On the other hand, breeders could opt for hand pollination using emasculated flowers (Sedgley et al. of these proceedings) which yields 100% hybrids but is labour intensive.

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Development of Hybridisation Techniques for *Acacia mangium* and *Acacia auriculiformis*

M. Sedgley*, J. Harbard*, R.M. Smith* and R. Wickneswari†

Abstract

The physiology of the flowers of *A. mangium* and *A. auriculiformis* was studied in relation to the development of a controlled hybridisation method. Both species showed pollen tube growth in the pistil, and penetration of ovules following hand self-pollination; and there was automatic self-pollination in unemasculated and unpollinated bagged flowers. Hybrid seed was produced following interspecific pollination in both directions and the hybrid status of the seedlings was confirmed using isozyme analysis. In vitro pollen germination was highly variable, and pollen lost in vitro germinability upon storage. Methods are described for the hybridisation of the two species involving hand emasculation and pollen transfer.

Acacia mangium and *A. auriculiformis* are two species, native to northern Australia, which have potential for timber and pulp production. There is also considerable interest in the hybrid between the two species, which appears to have arisen naturally in Sabah, and shows superior silvicultural characteristics over both parents. Attempts to conduct hand hybridisations between the two species have proved successful, but high levels of self-pollination have been detected in the progeny by isozyme analysis (Wickneswari et al. 1989). This is due to the fact that the flowers were not emasculated prior to cross-pollination (Gan and Sim 1987). Emasculation is normal practice prior to controlled hand pollination in species which are capable of selfing (Sedgley and Griffin 1989), but is very difficult in acacias due to the small size of the flowers. Controlled hybridisation is essential so that superior parents with desirable silvicultural characteristics can be crossed at will, and the aim of this research is to develop an improved, reliable method.

Acacia is a large genus, and most research has concentrated on the species native to temperate southern Australia (Sedgley 1987, 1989). The flowers of acacias are very small and are grouped into inflorescences. The pollen grains are characteristically grouped into polyads, composite structures which in *A. mangium* and *A. auriculiformis* consist of 16 grains. Self-incompatibility, a mechanism whereby the plant's own pollen will not produce seed set, has been reported in the genus. However, from the isozyme analysis results reported by Wickneswari et al. (1989), it appears that both *A. mangium* and *A. auriculiformis* show some degree of self-compatibility. This research investigates the structure and fertility of the flowers in relation to the development of efficient hybridisation methods.

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Materials and Methods

Plant material

Experiments were conducted on trees of both species in Australia and Malaysia (Table 1). Herbarium specimens of all experimental trees were lodged with the Australian National Herbarium in Canberra.

Pollinations

Spike-bearing branches on trees to be used as both female and male parent were labelled and bagged the day prior to anthesis (Fig. 1) when the flowers changed from green to yellow. Following anthesis of the first flowers, the bags were opened and all unopened buds were removed. The

Table 1. Experimental trees of *Acacia mangium* and *A. auriculiformis* at Atherton, Australia and Sabah, Malaysia.

Atherton		Sabah	
Tree number	Site	Tree number	Site
Acacia mangium			
1	Primary School	1–20	Brumas and Tawau
2	Primary School		
3	Cuda Park		
4	Cuda Park		
5	Cuda Park		
6	Cuda Park		
7	Tony Irvine's property		
8	Caravan Park		
9	Caravan Park		
10	Caravan Park		
11	Don Nicholson's property		
Acacia auriculiformis			
1	CSIRO Arboretum	1	SSSB Arboretum block 126D
2	CSIRO Arboretum	2	Tawau Golf Club
3	CSIRO Arboretum	3	Brumas Workshop compound
4	ACIAR plot	4	Taman Acacia, Bumas
5	ACIAR plot	5	Tawau Golf Club
6	Tony Irvine's property	6	Tawau Golf Club
7	Pine Street, Yungaburra	7	Tawau Golf Club
8	Cuda Park	8	Tawau Golf Club
9	Cuda Park		
10	Cuda Park		
11	Mt Windsor		
12	Rifle Creek		

open flowers (5–30 per spike, with a mean of 15) were subjected to one or more of the following treatments.

1. Unemasculated and unpollinated. The bagged flowers were not manipulated, and remained bagged until three days after anthesis.
2. Transfer of pollen using a test tube. Pollen was collected in a test tube, of similar diameter to the spike, by rubbing several spikes from the male parent at anthesis up and down inside the tube (Kenrick and Knox 1985). Open flowers of the female parent were emasculated by removal of all of the anthers using fine forceps. Pollen was transferred by brushing the emasculated flowers of the spike from the selected female tree against the walls of the tube.
3. Transfer of sieved pollen to unemasculated flowers using a paint brush. Spikes of the male parent were collected and air dried. The drying time varied with weather conditions. Dry spikes were sieved through a 53 micron sieve onto a dark surface. The pollen was collected with a fine paint brush and transferred to the stigma of unemasculated flowers.
4. Transfer of sieved pollen to emasculated flowers using a paint brush. Pollen prepared as in treatment 3, but applied to the stigmas of emasculated flowers.
5. Transfer of pollen to unemasculated flowers using the spike as a brush. Spikes from the selected male parent tree were brushed against the unemasculated flowers of the spike from the selected female tree (Gan and Sim 1987).
6. Transfer of pollen to emasculated flowers using the spike as a brush. As for treatment 5 but the flowers were emasculated.
7. Unbagged spikes were labelled and left unmanipulated for open pollination.

Both intraspecific and interspecific hand pollinations were conducted. Stigmas were observed following all stages with a magnifying lens for the presence of a polyad on the stigma, and the time taken to conduct treatments 3–6 was recorded. The spike-bearing branches were rebagged following all manipulations (Fig. 2) until three days following anthesis when the bags were removed. Flowers for microscopy were harvested at three days after pollination, fixed in Carnoy's fluid, and prepared for fluorescence microscopy for observation

of pollen tubes (Martin 1959). There were between 2 and 11 spikes per treatment. Flowers for seed set were checked at frequent intervals and the seed collected when mature. Seeds were germinated and the seedlings tested for hybrid status using isozyme analysis (Wickneswari et al. 1989).

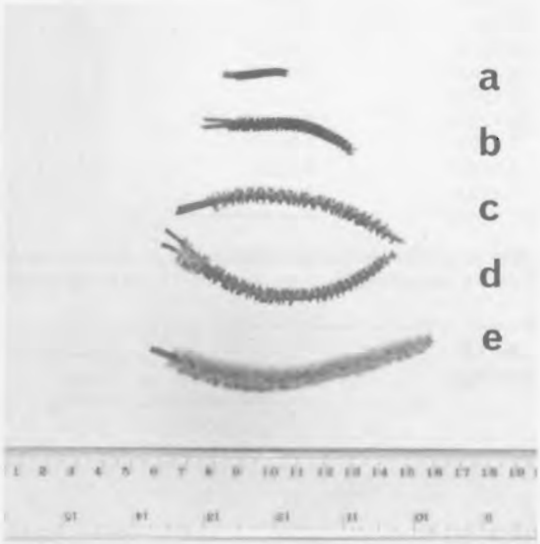

Fig. 1. Spikes of *Acacia mangium* at different stages of development: a, immature; b, one week prior to anthesis; c, one day prior to anthesis; d, anthesis of 10% of spike; e, anthesis of whole spike. Spikes are bagged for hybridisation at stage c.

Fig. 2. Bagged spike-bearing branches of *Acacia auriculiformis*.

Table 2. Average time taken for one pollinator to pollinate one spike of *Acacia auriculiformis* (Aa) using treatments 3–6 in Sabah in 1990.

Treatment (see text)	Tree	Time (minutes)
3	Aa 2	12.0
4	Aa 1, 2, 4, 6	12.4
5	Aa 1	8.2
6	Aa 4	6.7
Emasculated only	Aa 4	5.6

In vitro pollen germination

Pollen was germinated on a medium consisting of 20% sucrose, 1% agar, 0.01% boric acid, 0.03% calcium nitrate, 0.02 % magnesium sulfate and 0.01% potassium nitrate. Pollen was brushed onto the medium from fresh flowers, or from flowers which had been stored for varying lengths of time at 5° or 20°C, either fresh or following desiccation or freeze drying. A minimum of three replicates was set up for each sample, and a minimum of 100 polyads as scored per replicate. A grain was scored as germinated if the pollen tube was at least twice the length of an individual pollen grain.

Results

Floral morphology

The flower buds of *A. mangium* and *A. auriculiformis* were dark green during development, turning yellow on the day prior to anthesis. The flowers opened from the base of the spike in *A. mangium* (Fig. 1), and from the tip in *A. auriculiformis*, over the period of a single day. The flowers of *A. mangium* and *A. auriculiformis* had a similar morphology.

Pollinations

Treatments 5 and 6 were the quickest to conduct (Table 2), and emasculation of a spike took 5.6 minutes. Following emasculation no flowers had polyads on the stigma, and pollen transfer resulted in a mean of 54% of stigmas with a polyad. Using fluorescence microscopy, pollen tubes were clearly observed in the style and ovary of both species, and were also observed to penetrate the ovules (Table 3). Unpollinated and unemasculated flowers, which were bagged to prevent access to insects, had pollen tubes in the pistil due to automatic self-pollination (treatment 1). Comparison between the different pollen transfer methods following emasculation of the flowers showed little difference between methods 2 and 4 in terms of pollen tube growth. On average, between 30 and 40% of the pistils had a polyad on the

Table 3. Numbers of polyads and pollen tubes following emasculation and cross pollination of trees of *Acacia mangium* (Am) and *A. auriculiformis* (Aa) following different pollen transfer methods (mean ± S.E.).

Tree (see Table 1)	Treatment (see text)	Pistils with with a polyad (%)	Mean number of polyads on stigma	Mean number of pollen tubes in style	Mean number of penetrated ovules
Aa 1, 2, 6	1	9	0.1±0.1	0.4±0.1	0.2±0.0
Am 4 × Am 3	2	31	0.3±0.1	1.3±0.2	0.6±0.1
Am 4 × Am 8	2	33	0.3±0.0	1.7±0.3	0.7±0.1
Am 5 × Am 3	2	23	0.2±0.0	0.8±0.2	0.4±0.1
Am 6 × Am 5	2	32	0.3±0.0	1.4±0.3	0.6±0.1
Am 4 × Am 6	4	71	0.7±0.0	0.9±0.3	0.4±0.2
Am 6 × Am 5	4	18	0.2±0.0	1.0±0.3	0.3±0.1
Am 6 × Am 10	4	24	0.2±0.0	1.2±0.3	0.5±0.1
Aa 5 × Am 11	4	21	0.2±0.1	1.6±0.4	0.9±0.3

Table 4. Comparison of pollination methods for interspecific seed production of *Acacia mangium* and *A. auriculiformis*. Atherton results represent pooled data for crosses Aa 2, 8 and 9 × Am 3–6 and Am 5 and 6 × Aa 8–12, and Sabah results represent pooled data for crosses Aa 1–8 × Am 1–20.

Treatment (see text)	No. of spikes pollinated	No. of mature pods	Total no. of seeds	No. of seedlings	Hybrids (%)	Hybrid seedlings per pollinated spike	Time per hybrid seedling (min)
Atherton 1990							
2	71	25	177	111	100	1.48	–
3	3	0	–	–	–	–	–
4	13	0	–	–	–	–	–
Tawau 1990							
3	49	19	50	26	88	0.53	22.6
4	159	55	129	83	100	0.52	23.8
5	52	61	170	48	100	0.92	8.9
6	46	19	61	2	100	0.04	167.5
7	29	6	14	4	0	0.14	–

Table 5. In vitro pollen germination of fresh pollen of *Acacia anceps*.

Replicate number	Germinated polyads (%)	Percent polyads with *x* pollen tubes (%) *x*=							
		1	2	3	4	5	6	7	8
1	3	2	0	0	0	1	0	0	0
2	60	19	21	7	6	2	5	0	0
3	21	6	8	1	5	0	1	0	0
4	83	12	17	18	17	11	5	3	0
5	32	16	8	4	3	1	0	0	0
Mean ± S.E.	39.8 ±14.2	11.0 ±3.1	10.8 ±3.7	6.0 ±7.2	6.2 ±2.9	3.0 ±4.5	2.2 ±1.2	0.6 ±0.6	0

stigma which resulted in a mean of up to 0.9 penetrated ovules per ovary. This approximated to 13 penetrated ovules per spike based on a mean number of 15 flowers per pollinated spike. Ovule penetration was observed in the interspecific cross *A. auriculiformis* by *A. mangium*, as well as in the intraspecific crosses.

Most of the interspecific pollinations at Atherton in 1990 were conducted using treatment 2, the test tube method and emasculated flowers (Table 4). This method was successful in producing seed set. All of the seedlings were shown to be hybrid by isozyme analysis. A range of pollination techniques was compared in Tawau in 1990,

Table 6. In vitro pollen germination of stored pollen of trees of *Acacia mangium* and *A. auriculiformis* at Atherton (mean ± S.E.).

Age of pollen	Storage conditions	Germinated polyads (%)	Percentage polyads with x pollen tubes x =							
			1	2	3	4	5	6	7	8
Acacia mangium										
24 hours	Fresh 5°C	24.7 ±4.1	16.0 ±2.1	6.7 ±1.5	1.3 ±0.9	0.7 ±0.3	0			
4.5 hours	Desiccated 20°C	18.8 ±3.5	11.2 ±1.8	4.5 ±1.0	1.5 ±0.6	1.2 ±0.7	0.2 ±0.2	0	0.1 ±0.1	0
24 hours	Desiccated 20°C	1.6 ± 0.8	1.4 ±0.6	0.2 ±0.2	0	0	0	0	0	0
3 days	Desiccated 20°C	0	0	0	0	0	0			
2 months	Freeze dried	0	0	0	0	0	0	0	0	0
Acacia auriculiformis										
24 hours	Desiccated 20°C	12.8 ±5.8	8.7 ±3.9	1.7 ±1.2	1.4 ±0.6	0.8 ±0.5	0.3 ±0.3	0	0	0
6 days	Desiccated 20°C	0	0	0	0	0	0	0	0	0
6 weeks	Freeze dried	9.0 ±5.1	5.7 ±2.0	2.3 ±2.3	1.0 1.0	0	0	0	0	0
6 months	Freeze dried	2.8 ±1.2	2.2 ±0.9	0.4 ±0.3	0.2 ±0.2	0	0	0	0	0
10 months	Freeze dried	0.8 ±0.5	0.8 ±0.5	0	0	0	0	0	0	0
12 months	Freeze dried	0	0	0	0	0	0	0	0	0

including both emasculated (treatments 4, 6) and unemasculated (treatments 3, 5, 7) flowers. All methods resulted in seed set, but methods 3 and 7 (unemasculated treatments) produced a proportion of selfed seeds. Methods 2 and 5 gave the highest numbers of hybrid seedlings per pollinated spike, and method 5 was the quickest pollination method in terms of seed produced. All hybrid seedlings from the Atherton crosses conformed to the morphology described by Rufelds (1988). The seed from Sabah was very poor with low germination success, and the whole seedling was used for isozyme analysis.

In vitro pollen germination

The results of in vitro pollen germination were highly variable. A typical set of results involving the southern Australian species *A. anceps* is shown in Table 5. Germination percentage varied from 3 to 83%, even within the same experiment using pollen from the same inflorescence. Up to 7 of the 16 pollen grains of the polyad were observed to germinate. The overall mean from the experiment was 40% germination of the polyads, over half of which had only one or two grains germinated. Pollen of *A. mangium* produced 25% germination following storage for one day at 5°C but rapidly lost in vitro viability following desiccation or freeze drying (Table 6). Germination of *A. auriculiformis* pollen also decreased with increased time after storage, and the freeze-dried pollen had lost in vitro germination ability after 12 months in storage.

Discussion

This study has shown that hybrid seed of *A. mangium* and *A. auriculiformis* can be produced by pollen transfer using a variety of means. The simplest and quickest method is to brush together two spikes of unemasculated flowers. However, pollination of unemasculated flowers has resulted in the production of selfed seed in this and in other studies (Wickneswari et al. 1989). It is possible to remove the selfs in the nursery following detailed observation of the seedlings (Rufelds 1988), but this operation takes time and some selfs may be missed. This would result in mixed genotypes in the plantation. The most effective pollination method was the transfer of freshly collected, dry, sieved pollen using a fine paint brush to emasculated flowers. This ensured the collection of large amounts of pollen on the brush, thus increasing the probability of successful transfer to the stigma, and all methods using emasculated flowers resulted in 100% hybrid seed. Thus the seed producer must decide at which stage to spend the time, either at pollination or in the nursery, and what level of contamination by selfs is acceptable in the plantation. It is likely that the level of self-fertility, and thus the proportion of selfed progeny, will vary depending on the female parent.

Pollen tube growth was observed in the pistils and ovules of both species following intraspecific and interspecific pollination. Occasionally, more than one polyad was observed to adhere to a single stigma, and this may explain the observation by Wickneswari et al. (1989) of multiple parentage of some pods. Following hand pollination, 30–40% of pistils had a polyad which adhered and germinated resulting in 13 penetrated ovules per spike. There was then a reduction in potential seed number due to fruit shed and insect, fungal and climatic influences, resulting in a maximum of 1.5 seeds per spike.

Pollen storage is desirable for the crossing of trees which do not flower concurrently, or which are geographically separated. Methods for the testing of the viability of stored pollen are essential to ensure that time is not wasted using non-viable pollen. In vitro pollen germination was highly variable, and a minimum of three replicates was essential for reliable viability testing of *Acacia* pollen. Newman (1934) also reported variability in the germination of pollen of *A. baileyana* and that pollen taken between three and seven days after anthesis showed the highest viability. The pollen of *A. mangium* and *A. auriculiformis* did not store well as measured by in vitro pollen germination tests. However, pollen which had been freeze-dried and stored frozen for one month was successful in producing seed set, despite showing no pollen tube growth in vitro. Thus in vitro pollen germination tests are not a satisfactory measure of viability of *Acacia* pollen. Further research is required to develop improved storage methods for *Acacia* pollen, along with in vivo testing of viability.

From the practical point of view the most effective methodology to achieve 100% hybrid seed follows.

1. Select spikes as they change from green to yellow (Fig. 1).
2. Label and bag a group of spikes to be used as the female parent (Fig. 2). Single spikes should not be bagged as they are too fragile to support a bag. The window of the bag should face down to prevent buildup of condensation on the window.
3. Label and bag a group of spikes to be used as the male parent.
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.
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.
6. Any unopened buds are removed by gently rubbing the spike between the fingers.
7. The remaining florets (between 5 and 30 per spike) are then emasculated by removing the filaments from around the style using fine forceps.
8. The success of emasculations is measured by checking for contamination with self pollen on each stigma using a 30× lightscope.
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.
10. Sieve spikes through a 53 micron stainless steel sieve.
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.
12. Pollen is picked up with a fine brush with black hairs against which the pollen can be seen.
13. Transfer pollen to a stigma.
14. The presence of a polyad is assessed with the lightscope.
15. Spikes are rebagged.
16. The branches carrying the experimental spikes are labelled with numbered tags.
17. Remove the bags after three days.
18. Monitor pod development and harvest when mature.

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.

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

Any smooth black plastic surface against which the pollen can easily be seen after sieving.

Scissors — 5 cm blades.

Plastic labels.

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Mass Production of Hybrid Seed of *Acacia mangium* × *Acacia auriculiformis* in Biclonal Seed Orchards

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Abstract

If trees of *Acacia mangium* and *A. auriculiformis* are moderately self-sterile and have overlapping flowering periods, it should be possible to produce significant quantities of hybrid seed in clonal orchards. To test this option, eight experimental biclonal orchards were planted by Sabah Softwoods Sdn Bhd. Seven of these contained one clone of each of the parental species and in the remaining orchard the *A. mangium* clone was replaced by a spontaneous hybrid. The propagules used were rooted cuttings from marcottes on mature parent trees.

The orchards, which were planted between February and November 1990, are too young to have produced substantial information on seed production. However, preliminary conclusions are that: mature plus trees can be successfully propagated for orchard establishment; two-thirds of the *A. auriculiformis* clones (but none of the *A. mangium*) had started to flower within 11 months of planting; the hybrid clone had both flowered and produced viable seeds; and the earliest seeds were largely produced by self-fertilisation. To benefit from the work to date it will be necessary to continue observations for at least two more years.

SINCE *Acacia mangium* (AM) and *A. auriculiformis* (AA) will hybridise spontaneously in Sabah, and clonal propagation is technically feasible, it would be possible to make some worthwhile genetic improvement through a rigorous program of selection, clonal testing, and mass propagation of the best individuals. However, for progress in the long term, it is necessary to hybridise select genotypes from each of the parental species (Nikles and Griffin, these proceedings). The major aim of ACIAR Project 8630 has been to develop the various methodological procedures necessary to accomplish such a program (Razali et al., these proceedings).

Although we may wish to utilise advanced generation hybrids in future, the immediate means of capturing benefit from hybrid breeding will be through the planting of F_1 stock. The optimal procedure for producing bulk quantities of F_1 plants will vary according to the biology of the species, consideration of genetic gain and diversity, and the technical resources available for controlled crossing and propagation. Attributes which influence choice of seed or vegetative options are:

100% seed origin	100% vegetative origin
• small numbers required	• low cost, reliable vegetative propagation
• flowering reliable, early, synchronous across species	• long vegetative juvenile phase
• high self-incompatibility of at least one species	• flowering irregular
• controlled crossing cheap	• dissimilar requirements for flowering of each species
• vegetative propagation difficult/expensive	

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Assume that we wish to raise 1 000 000 plants; S denotes the number of different genotypes available; the annual vegetative replication rate per genotype is V. At one extreme, we may wish to select and clone 10 individuals by some combination of micro- and macro-propagation (S = 10, V = 100 000); at the other extreme, enough seed could be produced to raise the full complement as seedlings (S = 1 000 000, V= 1). Any intermediate position is possible.

For the AM × AA hybrid, the flowering and propagation biology is such that the economically optimal values for S and V are not immediately obvious. Vegetative propagation is easy; controlled pollination is difficult and expensive; but the breeding system and floral phenology are such that there seems a good prospect of mass producing hybrid seed by open pollination. This paper addresses the latter possibility, reporting establishment and preliminary results from the simplest possible hybridising orchard system ie. biclonal orchards containing 1 clone of each of the parental species.

Materials and Methods

Clones and propagation

In January 1989 fifteen 94-month-old trees were selected in a multi-provenance seedling seed orchard of AM at Brumas, Sabah, together with seven trees of 51-month-old AA in a trial established by FRC at Kolepis A, Sabah. Each selection was the best in its provenance on growth, form, and branching characteristics, as judged by the comparison tree method.

Vigorous branches about 3 cm in diameter were marcotted. After 40 days they were detached, pruned and potted up. Sprouts which developed on the marcottes were propagated as cuttings (Wong 1989) to produce a minimum of 25 plantable plants per clone.

Genotype determination

To facilitate assay of hybrid seed production in these experimental orchards, we wished to choose pairs of clones with different isozyme genotypes. Fresh foliage material from the candidate clones was sent to FRIM for analysis by starch gel electrophoresis methods described by Wickneswari (1989). As it turned out, the species show allelic differences at a number of loci and many genetically suitable pairings were available. Pairs were therefore finally selected on the basis of propagule availability. The genotypes of clones actually chosen for use in the orchards are given in Table 1. One of the clones (AM 483/34), included in orchard AM7D, was not part of the original set of selections and was not typed until after planting, when isozyme analysis confirmed morphological indications that it is, in fact, a spontaneous hybrid rather than pure *A. mangium*.

Experimental strategy

It will be important to plant operational biclonal orchards

Table 1. Clonal constitution of eight experimental biclonal orchards, showing isozyme genotypes of each clone at eight loci.

Orchard no.	Clone no.	Origin	Isozyme genotype							
			AAT-1	GDH-1	IDH-1	PER-1	PER-2	PGD-1	PGM-1	PGM-2
AM7B	AM137	Cardwell Qld	22	22	22	11	22	22	22	12
	AA1605	Iokwa PNG	?	11	22	12	22	13	22	11
AM7C	AM133	Mossman Qld	?	22	22	11	22	22	22	12
	AA1604	Balamuk PNG	?	11	11	12	11	13	11	22
AM7D	AM483/34*	Toko PNG	12	12	?	22	12	?	12	?
	AA1597	Balamuk PNG	?	11	13	22	11	13	12	22
AM7E	AM138	Mission Beach Qld	22	22	22	11	22	22	22	12
	AA1596	Balamuk PNG	?	11	11	12	12	13	12	12
AM7F	AM139	Mission Beach Qld	22	22	22	11	22	22	22	12
	AA1590	Bula PNG	?	11	11	22	11	33	12	11
AM7G	AM140	Mission Beach Qld	22	22	22	11	22	22	22	12
	AA1581	Balamuk PNG	11	11	11	22	12	33	12	22
AM7H	AM130	Daintree Qld	22	?	?	11	22	?	?	?
	AA1604	Balamuk PNG	?	11	11	12	11	13	11	22
AM7I	AM134	Mossman Qld	22	?	?	11	22	?	?	?
	AA1605	Iokwa PNG	?	11	22	12	22	13	22	11

* Natural hybrid.
? Genotype not determined.

with pairs of clones which have demonstrated synchronous flowering and cross-compatibility. Such information might be achieved by prior evaluation in an arboretum, or by planting multiple clones of each species and culling to leave the most favoured combination. These options were not available to us because of time constraints. We therefore decided to plant a series of small independent orchards, in the hope of finding at least a few matched combinations. This approach will also help determine the importance of clonal matching as a practical problem.

Design, establishment, and assessment

Between February and November 1990 a total of eight orchards were planted, using a sub-set of the AM clones and two of the AA clones twice (Table 1). Each orchard was established on a 7 × 7 m triangular spacing with alternating ramets of each clone and a total of 49 individuals (0.24 ha area), surrounded by a buffer of non-hybridising species. After establishment, the trees were hand-weeded as necessary and fertilised with CCM44 (12:6:22:3) at a rate of 100 g per tree.

At fortnightly intervals from October 1990 (9 months after planting the first trial), the presence of buds, flowers, and pods has been scored for each tree. A 1–3 scale was used to rate relative intensity of each attribute. Only the orchard AM7D, which contains the hybrid AM clone, has so far produced mature pods. These have been harvested, counted and numbers of seeds determined. The extracted seeds of the first harvest in January 1991 were sent to FRIM for isozyme analysis.

Results

Onset of flowering

In these species it is not unknown for inflorescence buds to initiate in the nursery on the first new flushes of growth made by marcotted plants. Early flowering in orchards was therefore anticipated. Within 10 months of planting, or less, four of the six AA clones had produced inflorescence buds (Table 2). By April 1991, the AA clones in the earliest planted orchards (AM7B,C,D) had also flowered, but only AA1597 in orchard AM7D had produced pods. To date, growth on the seven true AM clones has been solely vegetative.

Timing of flowering and pod-set

The number of trees in flower and bearing mature pods in AM7D (irrespective of intensity rating) is shown in Figure 1. Comparable data (not presented) are available for initiation and flowering of the AA clones in orchards AM7B,C,D.

The only 'AM' clone to have budded or flowered is the hybrid AM483/34 in orchard AM7D. This clone was in more or less continuous flower throughout the period from October 1990 to May 1991, never having less than nine trees with at least a light flower crop. The AA clone 1597 in AM7D showed a much clearer peak of flowering in January with another minor peak in April. We would therefore expect that the best prospect for hybrid seed would be from pods set in January.

Table 2. Planting dates, height growth and onset of first flowering for clones in each orchard.

Orchard no.	Month/year planted	Clone no.	Mean height (m)		First buds month/year	First flowers month/year
			at planting	6 months		
AM7B	2/90	AM137	0.53	0.85	–	–
		AA1605	1.46	2.47	10/90	1/91
AM7C	2/90	AM133	0.65	1.74	–	–
		AA1604	1.47	2.62	n.a.	10/90
AM7D	4/90	AM483/34*	0.63	1.43	n.a.	10/90
		AA1597	0.73	1.55	n.a.	10/90
AM7E	6/90	AM138	0.66	1.93	–	–
		AA1596	1.15	2.53	4/91	–
AM7F	6/90	AM139	0.68	2.03	–	–
		AA1590	1.19	2.70	–	–
AM7G	9/90	AM140	0.52	1.30	–	–
		AA1581	0.61	1.07	–	–
AM7H	9/90	AM130	0.53	1.38	–	–
		AA1604	0.57	1.59	–	–
AM7I	9/90	AM134	0.86	2.33	–	–
		AA1605	1.06	2.84	–	–

* Natural hybrid.
n.a. Actual date of bud production unknown

Quantities of pods and seeds in orchard AM7D

At each assessment period between January and April 1991, ripe pods were collected and seed extracted. During that time 23 of the AM ramets, and 19 of the AA clone, produced pods. Average pod and seed crops per ramet were very similar for the hybrid and the associated AA clone, at 22.8 pods/2.8 seeds per pod and 21.9/2.5 respectively (Table 3). The AA clone did however produce a greater proportion of its pods at the April harvest (Fig. 2), 18 ramets being harvested at that time cf. only 2 ramets in January. Comparable figures for the hybrid clone were 20 and 11 trees respectively.

Seed genotypes and selfing rates

In orchard AM7D, the first seeds produced by each clone were collected in January 1991 and sent for isozyme assay at FRIM.

Where we are able to assume that the male parent of any seed must have been either the self clone or the alternate species, and we know that the clones are homozygous for different alleles, any heterozygous seed must necessarily be hybrid.

Only the genotypes at the GDH-1 locus were scored. It was assumed that this would provide a simple discriminator of hybrid seed since, in earlier assays of samples from each species, AM was always fixed for allele 2 and AA for allele 1. As it turned out, the hybrid clone AM483/34 was actually heterozygous at this locus, so the progeny arrays in Table 4 cannot provide a direct measure of the frequency of backcrossing to the AA clone. We can however infer that this was very low by the alternative approach of estimating selfing rates, using the following logic:

- given that the GDH-1 genotype of AM483/34 is 12, selfing will produce progeny which segregate in the ratio of 1:2:1 for the 11,12,22 genotypes respectively;
- any 22 genotypes observed must be selfs since the AA clone is 11 and we are assuming no external pollen contamination; and
- since, in a population of selfed progeny of heterozygous parents, the 22 genotype has an expected frequency of 0.25, and in total we observed 21 seeds of this genotype, the estimated size of the selfed component of the 102 seeds assayed is $21 \times 4 = 84$.

The selfing rate estimate is therefore 84/102 = 82%.

Table 3. Production of pods and seeds by the two clones in orchard AM7D. Total of four monthly harvests Jan.–Apr. 1991.

Clone AM483/34			Clone AA1597		
Ramet no.	No. pods	Seeds/pod	Ramet no.	No. pods	Seeds/pod
1	8	2.0	2	3	0.3
3	5	5.2	4	40	2.6
5	10	1.6	6	21	2.7
7	11	2.5	8	17	3.1
9	0	–	10	0	–
11	32	2.7	12	0	–
13	13	4.5	14	0	–
16	23	3.0	15	18	6.1
18	23	2.1	17	20	1.6
20	27	2.4	19	39	2.5
22	21	3.3	21	42	1.7
24	52	2.4	23	33	3.9
26	5	2.2	25	29	2.9
28	38	2.5	27	5	1.8
29	6	2.7	30	54	4.4
31	24	3.8	32	5	0.2
33	22	2.6	34	18	1.9
35	31	3.2	36	0	–
37	25	2.1	38	6	2.0
39	40	2.4	40	133	3.3
41	51	3.1	42	0	–
44	8	2.4	43	5	1.6
46	0	–	45	29	1.9
48	72	3.0	47	4	4.2
			49	27	2.4
$\bar{x}$	22.8	2.8		21.9	2.6
SE $\bar{x}$	3.7	0.2		5.6	0.3

To test whether this differs significantly from 100% we can use a Chi^2 test for goodness of fit of the observed distribution of genotypes to the expected 1:2:1 ratio. $Chi^2_{2d.f.} = 2.52$, which is not significant at the 5% level.

For the AA seed the logic is simpler — we expect 50% of any hybrids to be of the 12 genotype (the other 50% being 11 and indistinguishable from AA selfs). In the small sample of 11 AA seeds assayed we found 1 hybrid seed, giving an estimated hybridisation rate of 18%.

Discussion

At this very early stage in the study we have shown that it is possible to propagate mature plus trees by marcotting and subsequent cuttings; and to obtain flowers on a majority of the AA clones within less than a year. We have also confirmed that at least the AA clone in orchard AM7D, and the hybrid clone 483/34, are capable of setting viable selfed seed. If AA clones 1604 and 1605 continue failing to set fruit in the absence of AM pollen, then this will be evidence that they are relatively more self-sterile.

None of the pure AM clones has yet flowered. According to the observations of Sedgley et al. (these proceedings), the best prospect for pod set in AM is from flowering in December–March and for AA flowering in May–September. Through the next year there will be an opportunity to verify whether this also applies to inter-specific crossing.

It is commonly found that propagules of forest trees do not set fruit or seed in a typical manner until they are vegetatively well established. We therefore expect to place greater weight on observations made from year two onwards in these bi-clonal orchards. It will be necessary to monitor for at least two consecutive years before we can draw firm conclusions about the effort needed for reproductive matching of specific pairs of clones. It is also critical to determine whether there is any general tendency for asymmetric production of hybrid seed between the parental species. If so, this would favour the idea of using one clone of the most cross-compatible species and several clones of the other which serve only as male parents (as has been done for the combination *E.grandis* × *Europhylla* by Aracruz in Brazil).

Results of other components of the Project 8630 work program will also influence the decision to proceed with hybridising orchards. In particular, we need to know whether the frequency of hybrid seed production varies with season (Wickneswari, these proceedings) and the success with which hybrid seedlings can be differentiated from intraspecific selfs in the nursery (Gan and Sim, these proceedings).

The concept of bi- or pauci-clonal hybridising orchards is not entirely new. A number of such orchards have been planted in Europe for the production of hybrid larch, with varying success (Paques 1989). An important observation was that particular combinations of clones hybridised

Table 4. Classification of seeds produced in the first harvest of pods January 1991) from orchard AM7D, according to genotypes at the GDH-1 loci. (Maternal genotype of AM483/34 is 12, and of AA1597,11).

Ramet no.	No. seeds	GDH-1 Genotype			Percentage detectable	
		11	12	22	Selfs	Hybrids
AM483/34						
3	8	5	3	0	0	
7	6	1	4	1	17	
11	5	5	0	0	0	
13	1	1	0	0	0	
22	10	0	7	3	30	
24	4	1	2	1	25	
26	1	0	1	0	0	
37	6	1	4	1	17	
41	7	0	5	2	29	
48	54	8	33	13	24	
All	102	22	59	21	20.6	n.a.
AA1597						
8	9	9	0	0		0
25	11	10	1	0		9.1
All	20	19	1	0	n.a.	9.1

successfully in some environments but not at others where flowering phenology was less synchronised (see also Sedgley and Griffin 1989). It may be important to make a careful choice of orchard location, and to screen flowering attributes of clones in that environment, if we wish to develop operationally reliable hybrid seed production programs for the *Acacia* species.

Acknowledgment

The cooperation of FRC Sepilok in permitting access to the *A. auriculiformis* trial at Kolapis, and in collecting some of the foliage samples used for isozyme analysis of candidate clones, is gratefully acknowledged.

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Nursery Identification of Hybrid Seedlings in Open Pollinated Seedlots

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Abstract

The accuracy of a seedling identification guide (Rufelds 1988) based on leaf taxonomic characteristics and development pattern was tested. Seedlings from separate seedlots of *Acacia mangium* Willd. and *Acacia auriculiformis* A. Cunn. ex Benth. parent trees growing in close proximity were raised in six independent nurseries under uniform conditions. The guide was then used to differentiate hybrid seedlings from those of the two parent types.

Consistent results were obtained in almost all the nurseries. However, the guide was biased and overestimated the proportion of hybrids by a mean of 9% and 12% for the *A. mangium* and *A. auriculiformis* seedlots respectively. This was validated by comparison with results from isozyme analysis of the seedlots. Problems were encountered in the use of the guide. These included subjectivity, tediousness and the uselessness of most taxonomic characteristics. Due to the excessive amount of time needed to assess each seedling, the method was considered impractical and too costly for implementation in a commercial nursery.

A proposed simplification of the guide which necessitates the observation of only two leaf characteristics was shown to give a small improvement in accuracy. More importantly, it is objective, simple and practical. Also, it allows earlier identification of the seedlings and is independent of provenance effects.

A hybrid of *Acacia mangium* Willd. and *Acacia auriculiformis* A. ex Cunn. Benth. was first discovered in Sabah, Malaysia in the late 1970s (FAO 1982). These hybrid trees in Ulu Kukut were observed to display superior size, branch form and circularity of cross-section to the neighbouring *A. mangium* trees (Rufelds 1987). Furthermore, there were indications that it had wood density and strength properties slightly superior to those of *A. mangium* (Rufelds 1987). As a result, interest was generated in investigating the potential and practicality of using the hybrid for reforestation.

In a hybrid program, the planting materials may be obtained from hybrid seedlings or from hybrid propagules. Seed collections can be undertaken in the field where natural hybridisation is occurring or from controlled pollination. In controlled pollination, emasculation of the *Acacia* flowers is difficult due to their small size. The easier option for mass producing the hybrids using a hybridisation orchard is under investigation. Such orchards, planted with mixtures of either selected seedlings or clones of each species, are expected to produce seed which is a mixture of hybrids and inter-specific crosses or selfed. In order to make use of the desirable hybrid seedlings, it is essential to have a technique by which the nursery staff are able to identify them.

There are several possible methods that can be used to identify the *Acacia* hybrids. Isozyme analysis and examination of the extra-floral nectories are complex, require expertise and sophisticated equipment. A less complicated method would be the use of morphological keys by which nursery staff could quickly differentiate the seedlings.

Work completed at the Forest Research Centre (FRC), Sepilok by C.W. Rufelds (1988) indicated that it was possible to differentiate seedlings of *A. mangium*, *A. auriculiformis* and their hybrids on the basis of leaf development, morphology and taxonomic characteristics.

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As a result of this work, a guide for distinguishing the seedlings was developed. This guide consists of two parts. The first part is a Leaf Taxonomic Characteristics table (LTC) which consists of taxonomic and quantitative characteristics. These characteristics include the pinnule margin pubescence, the colour of the margin and underside of the pinnule, as well as the phyllode morphology. The second part of the guide is a Leaf Development Pattern (LDP) table which comprises essentially the developmental sequences of the different pinnate leaves and phyllode.

Although the Rufelds seedling guide was subsequently tested in the FRC nursery and found to be useful, it had never been tried elsewhere. The aim of this component of the ACIAR project was therefore to confirm or improve the method by testing it in a number of independent nurseries and verify it using isozyme analysis.

Materials and Methods

Establishment

The experiment was conducted in six nurseries at Sabah Forest Industries Sdn Bhd (SFI), Forest Research Centre (FRC), Sabah Softwoods Sdn Bhd (SSSB), Luasong Forestry Centre (Innoprise), Sabah Forestry Development Authority (SAFODA) and Forest Research Institute Malaysia (FRIM).

Seed for the experiment was supplied by FRIM. For *Acacia mangium* seedlots, 500 seeds from each of four parent trees were bulked and divided into portions of 250 seeds per portion. For *A. auriculiformis* seedlots, 400 seeds from each of five parent trees each were bulked and also divided into portions of 250 seeds per portion. These seedlots were then distributed to the participating nurseries. At FRIM, samples of the seed were also assayed to determine the percentage of hybrids by isozyme analysis.

At each nursery, the seed was pretreated with hot water at 100°C for a half minute and then allowed to soak in tap water for 24 hours. The seed was then sown on a moist paper towel and kept in ambient conditions of light and temperature. The seed started to germinate in the first week after sowing and the germinated sprouts were transplanted into polybags. The polybag mixture consisted of top soil supplemented with CIRP at 5 kg/m^3 of soil. The seedlings were kept under 50–70% shade and watered twice a day. To help prevent fungal infection, fungicide was applied to the seedlings every 10 days.

Assessments

Weekly assessments were made on each seedling until the onset of the full phyllode which typically took up to more than 10 weeks in the case of *A. mangium* seedlings. The Rufelds seedling guide was applied to differentiate the seedlings into the different taxa.

Results and Discussion

Rufelds' method — utilisation of both LTC and LDP

Hybrid percentage

The results obtained at each nursery through the assessments of both the leaf development pattern (LDP) and leaf taxonomic characteristics (LTC) are summarised in Table 1 and Figs 1, 2.

Table 1. Rufelds' method — identification results.

Nursery	*Acacia mangium* parent				*Acacia auriculiformis* parent			
	Total no. of seedlings	No. of hybrid seedlings	Hybrid (%)	Overestimate (%)	Total no. of seedlings	No. of hybrid seedlings	Hybrid (%)	Overestimate (%)
SFI	61	25	41.0	3.3	12	4	33.3	6.8
FRC	83	35	42.2	4.5	–	–	–	–
SSSB	112	48	42.8	5.1	136	50	36.8	10.3
ICSB	83	37	44.6	6.9	169	62	36.7	10.2
SAFODA	56	34	60.7	23.0	–	–	–	–
FRIM	87	41	47.0	9.3	100	49	49.0	22.5
Average			46.4	8.7			39.0	
Isozyme analysis			37.7				26.5	
Computed t			2.89*				3.62*	

Note: 1. No results were available from FRC and SAFODA for *A. auriculiformis* seedlots.
2. Computed *t* for difference between nursery and isozyme analysis results. (* < 0.05; ns – not significant).

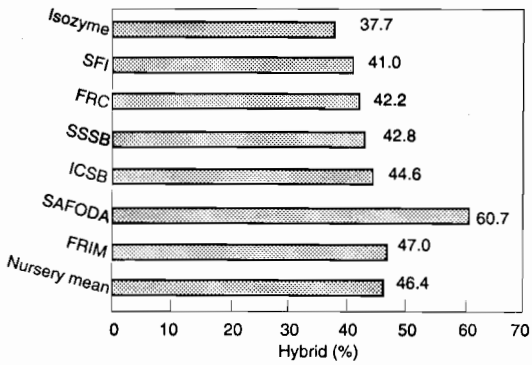

Fig. 1. Rufelds' method — hybrid percentage for *Acacia mangium* seedlot

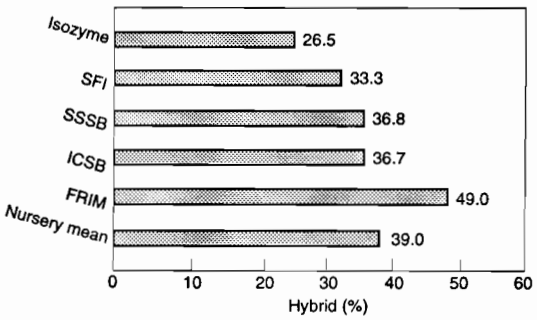

Fig. 2. Rufelds' method — hybrid percentage for *Acacia auriculiformis* seedlot

The percentage of seedlings identified as hybrids was fairly consistent among all the nurseries. The exceptions were SAFODA (for seedlings from *A. mangium* seedlots) and FRIM (for seedlings from *A. auriculiformis* seedlots). For the *A. mangium* seedlots, all the nurseries reported between 41% and 47% as hybrid except SAFODA which obtained 60.7%. In the case of *A. auriculiformis* parent trees, most nurseries reported 33.3% to 36.8% of seedlings as hybrid with only FRIM reporting a higher percentage of 49%. Due to poor germination of the *A. auriculiformis* seedlots for FRC and SAFODA, no results were available from those two nurseries.

Comparison of the nursery results with those from isozyme analysis indicated that the seedling guide tended to give slight overestimates in the hybrid per cent. The percentages of hybrids from isozyme analysis were 37.7% and 26.5% for the *A. mangium* seed lots and *A. auriculiformis* seedlots respectively. When subjected to the t-test, the nursery results were significantly different from the isozyme analysis results at the 0.05 level. Hence the guide produced statistically significant biased results. On the average, the overestimate was higher for the *A. auriculiformis* seedlots than the *A. mangium* seedlots.

Time period

In Table 2, the leaf position and time period for the onset of the first full phyllode are given. The results indicated that, for the hybrid seedlings, the onset of the first full phyllode occurred mostly at the 5th to 9th leaf position, usually in 6 to 9 weeks after transplanting into polybags. For *A. auriculiformis* seedlings, the onset was earlier being mostly in 4 to 6 weeks at the 3rd to 5th leaf positions. *A. mangium* seedlings, on the other hand, for the most part only developed the full phyllode from the 10th week onwards.

The results therefore showed that, by using both the LTC and LDP, the identification process would take up to more than 10 weeks before the different seedling types could be confirmed.

Problems

Assessment method. Problems in assessment were encountered in using the seeding guide, especially the LTC part.

In the LTC table, assessors were required to base their assessments on the colour and pubescence of the pinnule margin, and the colour of the pinnule underside, as well as the phyllode features. Among these characteristics, pinnule colour was found to be too subjective and could not be reliably applied. Assessors reported difficulties in deciding on the colours and the decisions of different individuals were not consistent.

Table 2. Onset of the first full phyllode.

Nursery	Leaf position for the onset of first full phyllode				No. of weeks taken for the onset of first full phyllode			
	A. mangium	*A. auriculiformis*	*A.m.* hybrid	*A.a.* hybrid	*A. mangium*	*A. auriculiformis*	*A.M.* hybrid	*A.A.* hybrid
SFI	9–11	4–5	5–8	5–7	10–14	5–6	6–10	6–8
FRC	10–?	–	6–9	–	9–?	–	6–9	–
SSSB	–	4–5	5–8	5–7	–	4–5	6–9	5–7
ICSB	8–11	3–5	6–8	5–9	9–14	5–9	8–10	6–9

Table 3. Shape ratio (length/width) of phyllode.

Nursery	*A. mangium*		*A. auriculiformis*		*A.m.* hybrid		*A.a.* hybrid	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
SFI	3.0–5.3	3.9	5.6–10.2	5.2	4.5–10.0	6.2	5.6–9.1	6.8
FRC	3.0–3.4	3.2	–	–	4.1–10.8	5.8	–	–
SSSB	–	–	5.5–16.2	9.5	3.6–9.2	5.9	3.7–12.4	5.2
ICSB	3.4–6.4	5.1	6.1–22.0	11.1	4.0–11.3	7.5	4.5–9.0	6.8

Note: From a small sample of a few seedlings only.

The leaf colours were also observed to change with age. As the seedling grew older, it tended to lose its red colour. Furthermore this characteristic also varies with provenance (Rufelds 1988).

The problem of subjectivity also arose in the case of pinnule pubescence. Pubescence was not an appropriate characteristic for differentiating between *A. mangium* and hybrids. However, it was useful in differentiating *A. auriculiformis* from *A. mangium* and their hybrids.

The difference between the shape of the phyllode of the hybrid seedling and those of the parents was found not distinct enough for easy identification. Even with actual measurements of the shape ratio, this trait was still not an accurate index. The range of shape was too wide as indicated in Table 3. Other features such as the number of veins and the phyllode texture were also not reliable.

By comparison, the leaf development pattern (LDP) was reported by most assessors to be more useful for differentiating the two species and their hybrids.

Assessment time and cost. Another problem with the Rufelds' guide was the large amount of time taken to assess each seedling. Typically, it took an average of 1.5 hours to assess just 100 seedlings. Thus, one nursery worker could be expected to assess only 500 to 600 seedlings each day (8 hours work). In a commercial nursery where millions of seedlings are involved, the method would be impractical.

With the amount of time needed for identification, the method proved to be costly. Assuming an average wage of $8.00 per day for each nursery staff, the operation would add an extra cost of 1.5 cent to each seedling assessed. Depending on the proportion of hybrids in the seed, this additional cost would be much higher for the actual seedlings eventually utilised for planting (i.e.. hybrid seedlings).

Observations of specific characteristics

(For this section, see Appendix 1, Description of leaf parts; Appendix 2, Leaf Development Pattern Table; and Appendix 3, Leaf Taxonomic Characteristics Table.)

Method A — Number of pinnate leaves per seedling

According to the LDP table, *A. mangium* seedlings develop the highest number of true leaves or pinnate leaves before the onset of the full phyllode. On the other hand, *A. auriculiformis* seedlings have the lowest number of pinnate leaves. As such, it was possible to differentiate the seedlings based on the number of pinnate leaves they developed regardless of the types. This is evident from the results given in Table 2.

The use of this procedure (method A) resulted in a slightly lower hybrid per cent compared with Rufelds' method (Tables 4, 5 and Figs 3, 4). The percentage of seedlings identified as hybrids was closer to the isozyme analysis. For the *A. auriculiformis* seedlots, the difference between the nursery results and isozyme analysis was not significant at the 0.05 level as indicated by the t-test.

From the results, it was inferred that this simpler method produced a slightly improved accuracy. Furthermore, it was less tedious and identification could be accomplished earlier, from 6 to 10 weeks after transplanting.

Method B — 4-pinn leaf or number of 2-pinn leaves (A. mangium *seedlot)(see Appendix 1)*

In this method, any seedling with a 4-pinn leaf or, with 5 or more 2-pinn leaves, was classified as *A. mangium.* This method produced results comparable to those by Rufelds' method (Table 6 and Fig. 5). The new method was very simple and did not entail any subjective assessment. Also, this method would enable the seedling types to be differentiated before 10 weeks.

Method B — 2nd leaf pinnule margin pubescence (A. auriculiformis *seedlot)*

As *A. auriculiformis* seedlings lack pubescence on their pinnule margin, a quick way to segregate the seedlings would be to observe the presence or absence of such pubescence. Based on this method, a slightly higher mean percentage of hybrids was obtained compared with results from using both the LTC and LDP methods (Table 7 and Fig. 6).

Table 4. Method A — results based on the number of pinnate leaves before emergence of full phyllode (A. *mangium* parent).

Nursery	No. of seedlings with < 8 pinnate leaves (i.e. hybrid)	Hybrid (%)	Overestimate (%)	No. of weeks before identification possible
SFI	26	42.6	4.9	6–10
FRC	32	38.6	0.9	6–10
SSSB	46	41.1	3.4	6–10
ICSB	35	45.4	7.7	8–12
SAFODA	34	49.3	11.6	8–9
Average		43.4	5.7	
Computed t		3.09*		

Note: 1 No raw data available from FRIM
2 Computed t for difference between nursery and isozyme results
(* < 0.05; ns – not significant)

Table 5. Method A — results based on the number of pinnate leaves before emergence of full phyllode (*A. auriculiformis* parent).

Nursery	No. of seedlings with < 8 pinnate leaves (i.e. hybrid)	Hybrid (%)	Overestimate (%)	No. of weeks before identification possible
SFI	3	25.0	–1.5	5 – 6
SSSB	46	7.3	7.3	5 – 7
ICSB	45	30.8	4.3	5 – 5
Average		29.9	3.4	
Computed t		1.30*		

Note: 1 Computed t for difference between nursery and isozyme results (* < 0.05; ns – not significant).

Table 6. Method B — results based on the presence of 4-pinn leaf or number of 2-pinn leaves (*A. mangium* parent).

Nursery	No. of seedlings with no 4-pinn leaves or < 5 2-pinn leaves (i.e. hybrid)	Hybrid (%)	Overestimate (%)	No. of weeks before identification possible
SFI	27	44.3	6.6	6–9
FRC	34	41.0	3.3	6–9
SSSB	46	44.1	3.4	6–9
ICSB	34	44.2	6.5	7–10
Average		42.6	5.0	
Computed – t		5.35**		

Note: 1 No raw data available from FRIM; erroneous recording in SAFODA data.
2 Computed t for difference between nursery and isozyme results
(** < 0.01; ns – not significant).

Moreover, since the presence or absence of the pinnule margin pubescence could be observed in the second leaf, the method therefore enabled very early identification viz. within 2–3 weeks of transplanting. Compared with Rufelds' method, it was both simple and objective, requiring the observation of only a single trait.

Simplified method

In Figures 7–8, comparisons of the mean results obtained by the different procedures are illustrated. Results from the combined use of Methods A and B are also included. From these comparisons, it is evident that Methods A

Table 7. Method B — results based on the presence of pubescence on the pinnule margin of the second leaf(*A. auriculiformis* parent).

Nursery	No. of seedlings with pubescence on second leaf pinnule margin i.e. hybrid)	Hybrid (%)	Overestimate (%)	No. of weeks before identification possible
SFI	5	41.6	15.1	2–3
SSSB	59	43.4	16.9	2–3
ICSB	47	36.1	9.6	2–3
Average		40.4	13.9	
Computed t		6.31**		

Note: 1 Computed t for difference between nursery and isozyme results (** < 0.01; ns – not significant).

Table 8. Method A and B combined — identification results.

Nursery	*Acacia mangium* parent			*Acacia auriculiformis* parent		
	No. of hybrid seedlings	Hybrid (%)	Overestimate (%)	No. of hybrid seedlings	Hybrid (%)	Overestimate (%)
SFI	26	42.6	4.9	3	25.0	–1.5
FRC	32	38.6	0.9	–	–	–
SSSB	46	41.1	3.4	46	33.5	7.3
ICSB	34	44.2	6.5	47	27.5	1.3
Average		41.6	3.9		25.9	2.4
Isozyme analysis		37.7			26.5	
Computed t		3.29*			0.91 ns	

Note: 1 Computed t for difference between nursery and isozyme results (* < 0.05; ns – not significant).

Table 9. The simplified method.

Parent type	Approx. assessment time (weeks from transplanting)	Seedling characteristics	
		Non-hybrid	Hybrid
Acacia mangium	9	Presence of 4-pinn leaf or 5 or more 2-pinn leaves	Absence of 4 pinn leaf or less than 5 2-pinn leaves
	10	8 or more pinnate leaves before onset of full phyllode	Less than 8 pinnate leaves before onset of full phyllode
Acacia auriculiformis	3	Presence of pubescence on second leaf pinnule margin	Absence of pubescence on second leaf pinnule margin
	7	5 or more pinnate leaves before onset of full phyllode	Less than 5 pinnate leaves before onset of full phyllode

Note: 1 Seedlings should be kept under 50–70% shade.
2 Pinnate leaf includes all leaf types except the full phyllode and cotyledons.

and B, when used together, would enable a more accurate identification of the hybrid seedlings. This technique produced results closer to that of the isozyme analysis (Table 8 and Figs 9,10); and, in the case of the *A. auriculiformis* seedlot, no significant difference at the 0.05 level was detected by the t-test.

Based on the improved accuracy, a simplified identification method comprising both methods A and B is proposed. This method is given in Table 9 and Figs 11,12. It involves two stages of assessment. In the first assessment, the majority of the seedlings are segregated. The second assessment is done at a later stage to pick up the

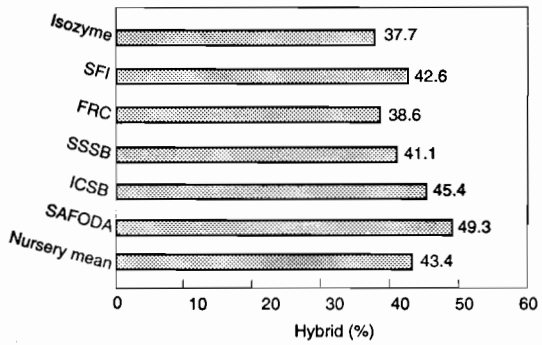

Fig. 3. Method A — hybrid percentage for *Acacia mangium* seedlot

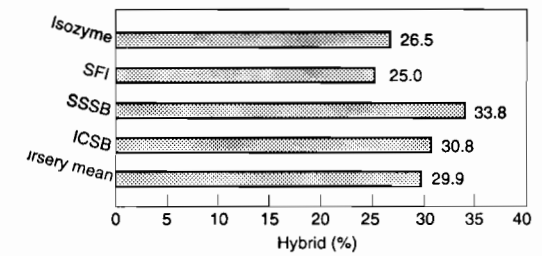

Fig. 4. Method A — hybrid percentage for *Acacia auriculiformis* seedlot

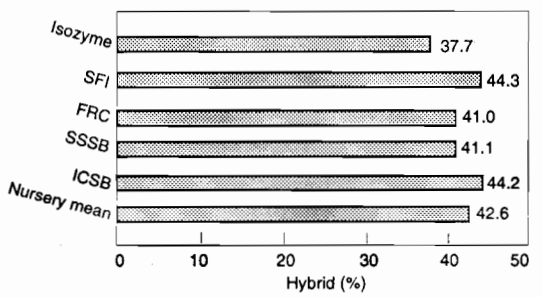

Fig. 5. Method B — hybrid percentage for *Acacia mangium* seedlot

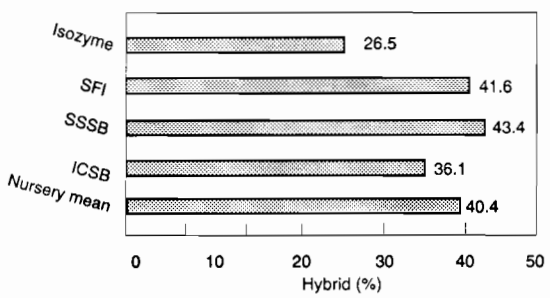

Fig. 6. Method B — hybrid percentage for *Acacia auriculiformis* seedlot

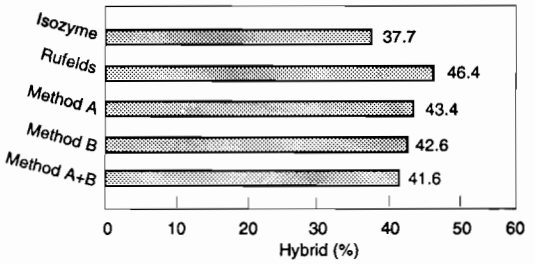

Fig. 7. Mean hybrid percentage, by method, for *Acacia mangium* seedlot

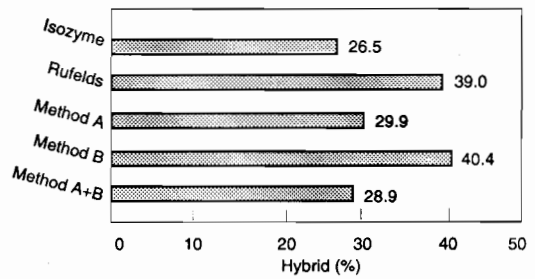

Fig. 8. Mean hybrid percentage, by method, for *Acacia auriculiformis* seedlot

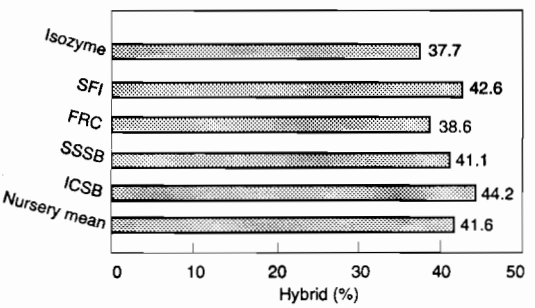

Fig. 9. Method A + B — hybrid percentage for *Acacia mangium* seedlot

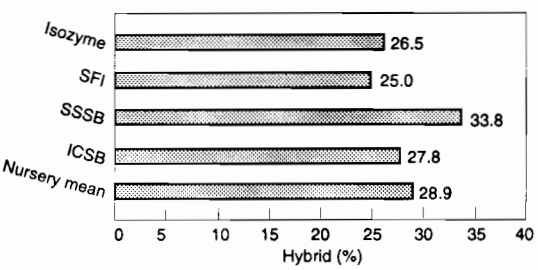

Fig. 10. Method A + B — hybrid percentage for *Acacia auriculiformis* seedlot

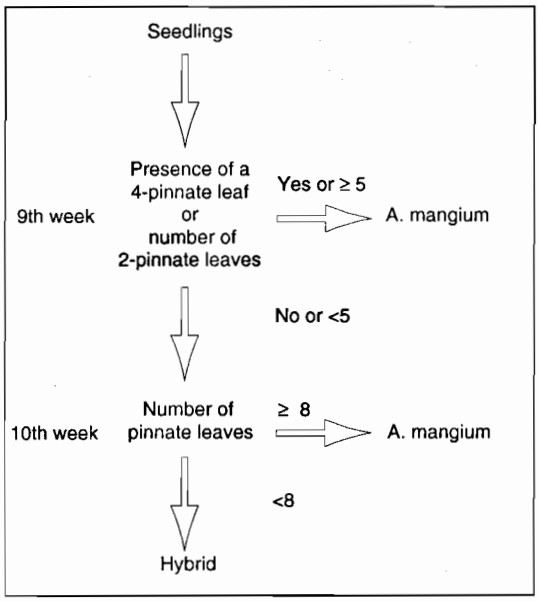

Fig. 11. Diagrammatic representation of the simplified method (*Acacia mangium* parents)

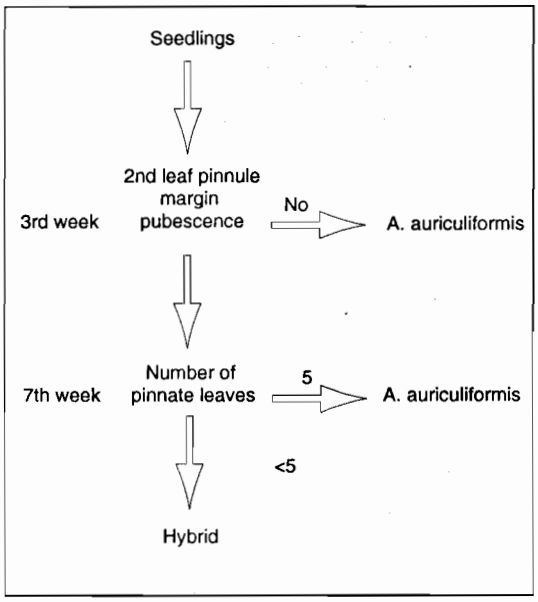

Fig. 12. Diagrammatic representation of the simplified method (*Acacia auriculiformis* parents)

remaining seedlings which could not be identified earlier on. For *A. mangium* seedlots, due to the short interval between assessments, the first assessment could be done at the time of the second assessment to minimise assessment time. This simplified method is based on only two characteristics. For seedlings from *A. mangium* parents, the two characteristics are :

- the presence of a 4-pinn leaf or the number of 2-pinn leaves, and
- the number of pinnate leaves.

For *A. auriculiformis* parent seedlots, the two characteristics are:

- the second leaf pinnule margin pubescence, and
- the number of pinnate leaves.

Since the method entails the observation of only two traits, it is simpler and less time-consuming than Rufelds' method. It is objective and offers a slight improvement in accuracy. It is also possible to differentiate the seedlings much earlier.

Moreover, as the method is based on the leaf development pattern, and pubescence or otherwise in the case of *A. auriculiformis*, it could be used regardless of provenance. In the study by Rufelds (1988), it was reported that there was no significant difference in the seedling leaf development pattern between provenances. The lack of pinnule margin pubescence in *A. auriculiformis* seedlings was also found to be unaffected by provenance from the author's own observations of seedlings from various provenances.

Conclusion

The results of the experiment indicated that the Rufelds' seedling guide was useful for the identification of the hybrid seedlings from the two parent types. It produced consistent results at almost all the six nurseries. In most nurseries, the percentages of hybrids in separate seedlots from *A. mangium* and *A. auriculiformis* parents were determined to be in the range of 41%–47% and 33.3%–36.8% respectively. However, when compared with the results from isozyme analysis (37.7% for the *A. mangium* seedlot and 26.5% for the *A. auriculiformis* seedlot), it was found that there was a tendency for the seedling guide to give a slight overestimate of approximately 10% in the hybrid percentage. This positive bias was statistically significant at the 0.05 level.

Although the Rufelds' method was effective, difficulties were encountered in using it. Its major weakness was the subjectivity associated with assessing many of the leaf characteristics. Making the right decision in the assessment of these characteristics was reported by all assessors to be the most difficult.

Most of the leaf taxonomic characteristics in the guide were found to be useless and confusing to the assessors. The pinnule colour was difficult to use as a key and was

not accurate. Furthermore, it tended to change with age. The phyllode shape, its texture, as well as the number of veins, were also found to be inaccurate or useless as indexes. Pinnule pubescence was also a poor index for distinguishing seedlings of *A. mangium* and its hybrids. However, it proved to be a useful index for the differentiation of *A. auriculiformis* seedlings from both its hybrids and *A. mangium*.

Another problem with the Rufelds' method was that it required a lot of time for assessment. As such, it was impractical and would be too costly to be used in a commercial nursery.

A simplified method was found to give slightly improved accuracy. It is simple and entails the observations of only two leaf characteristics:

- The presence or absence of second leaf pinnule margin pubescence and the number of pinnate leaves for *A. auriculiformis* seedlots.
- The 4-pinn leaf or the number of 2-pinn leaves, and the number of pinnate leaves, for *A. mangium* seedlots.

This simplified method is objective and less time-consuming. More importantly, it is also independent of provenance differences.

Acknowledgments

We would like to thank the various organisations that collaborated in this study. They include FRC, SSSB, ICSB, SAFODA and FRIM. The support given by Dr Rod Griffin of CSIRO is also acknowledged.

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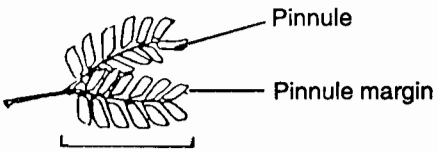
Appendix I

Description of Leaf Parts

Bipinnate Leaf

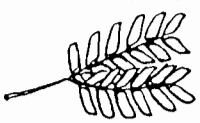


Pubescence = white hairs l = length w = width

Description of leaves:

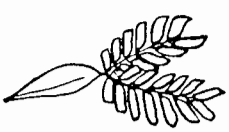
Once-pinnate

Bipinnate (2-pinn)

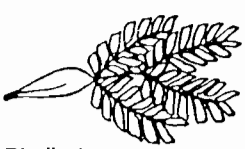


Compoundly pinnate leaf with four pinnae (4-pinn)

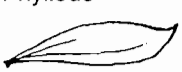
Phyllode leaf with a bipinnate leaf attached to apex (Phyllode + 2-pinn)



Phyllode with a compoundly pinnate leaf with four pinnae attached to apex (Phyllode + 4-pinn)



Phyllode



Appendix II

Leaf Development Pattern Table

Approx. leaf no.*	Leaf type		
	A. mangium	Hybrid	*A. auriculiformis*
1st	once-pinnate	once-pinnate	once-pinnate
2nd	2-pinn	2-pinn	2-pinn
3rd	2-pinn	2-pinn	Phyllode+2-pinn
4th	2-pinn	2-pinn or Phyllode+2-pinn	Phyllode+2-pinn or Phyllode
5th	2-pinn or 4-pinn	Phyllode+2-pinn or 2-pinn	Phyllode
6th	4-pinn or 2-pinn	Phyllode+2-pinn or Phyllode+2-pinn or Phyllode	Phyllode
7th	4-pinn	Phyllode or Phyllode+2-pinn or Phyllode+4-pinn	Phyllode
8th	4-pinn or Phyllode+4-pinn or Phyllode+2-pinn	Phyllode	Phyllode
9th†	Phyllode+4-pinn or 4-pinn or Phyllode+2-pinn	Phyllode	Phyllode
10th	Phyllode+4-pinn or Phyllode	Phyllode	Phyllode
11th	Phyllode+4-pinn or Phyllode	Phyllode	Phyllode
12th	Phyllode	Phyllode	Phyllode

* Climatic conditions (wet or dry season) will have an effect on the growth rate and development of *Acacia* seedlings. Therefore the leaf no. is an approximate value which is subject to variation. The overall sequence of leaf emergence should remain the same.

† *A. mangium* seedlings will occasionally exhibit Phyllode+6Pinn leaves at this stage.

(Source: Rufelds 1988)

Appendix III

Leaf Taxonomic Characteristics Table

Leaf type/characteristic	Trait*		
	A.mangium	hybrid	*A. auriculiformis*
Once-Pinnate			
Pinnule margin colour	red	red to green	green
Pinnule margin pubescence	dense	moderate	none
Pinnule underside colour	red	pinkish-red to green	green
Bipinnate			
Pinnule margin colour	dark red	pinkish-red to green	green
Pinnule margin pubescence	dense	moderate	none
Pinnule underside colour	red to reddish-green	reddish-green to green to light green	green to light green
Phyllode + 2-pinn			
Pinnule margin colour	red	red to green	green
Pinnule margin pubescence	dense	dense to moderate	none
Phyllode			
Shape	ovate	more elongate	elongate
Shape ratio (length:width)	2.5–3.4	3.6–5.9	8.8–11.3
No. of veins	3–4	2–3	1–2
Texture	leathery	membraneous	membraneous

* When assessing the trait or range of traits (from most common to least common) it is important that assessments for a characteristic on one seedling are made *relative* to the appearance of the same characteristic on other seedlings.

Techniques for Starch Gel Electrophoresis of Enzymes from Acacias

R.Wickneswari* and M. Norwati*

ISOZYME analysis has been extensively used over the past several decades in investigations of the genetics of a large number of organisms from fruit flies and humans to crop plants. Investigations into the genetic diversity and breeding systems of some *Acacia* species using isozyme analysis have been carried out recently. These include studies on *A. albida* (Joly and Zeh-Nlo, pers. comm.), *A. auriculiformis* (Moran et al. 1989a, Wickneswari and Norwati 1991), *A. crassicarpa* (Moran et al. 1989a) *A. holosericea* (Moran, pers. comm.), *A. mangium* (Moran et al. 1989b) and *A. melanoxylon* (Playford et al. 1991). Some of these *Acacia* species, viz. *A. mangium*, *A. auriculiformis* and *A. holosericea*, are becoming increasingly important in afforestation, reforestation and agroforestry programs in Southeast Asia, Africa, China and India. Hence, this information on the genetics of these species would be useful in designing tree breeding programs for them.

What are isozymes?

Isozymes are different molecular forms of the same enzyme with the same substrate specificity but different electrophoretic mobility (Markert and Moller 1959). These different molecular forms of enzymes arise from mutations which may occur as a result of changes in the base sequence of DNA molecules. A change in this sequence may result in a change in the amino acid sequence of polypeptide chains (Fig. 1). After a mutation of this type, the protein or enzyme formed from DNA translation will differ slightly from the one produced before the mutation. Although some mutations can be catastrophic, many mutations merely result in a slight change in enzyme structure without any adverse effect on function.

The new base sequence producing the slightly altered enzyme forms part of a gene which is the segment of a chromosome or DNA molecule that produces a single protein product. Different forms of a gene that produce structurally altered proteins are termed alleles. In the case of genes coding for enzymes, the structurally different enzymes produced by different alleles are termed allozymes. The different allozymes of an enzyme are referred to as isozymes. When the genetic change from a gene mutation results in allozymes having different surface charges because of amino acid substitutions on the surface

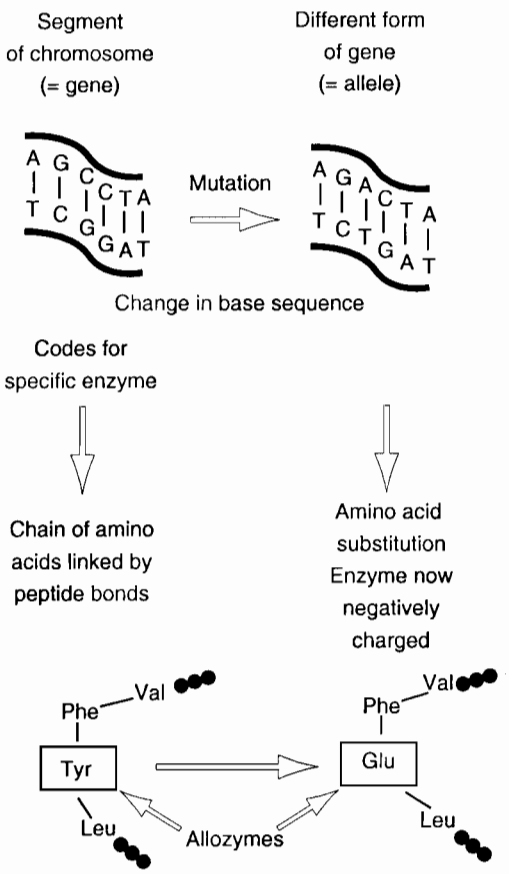


Fig. 1. Diagrammatic representation of enzyme synthesis and origin of genetic variation leading to the formation of allozymes.

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of the enzyme molecule, the allozymes will move at different rates in an electric field. Therefore electrophoresis of enzymes can indicate genetic differences originating from gene mutations. In this way, basic genetic differences among individuals or populations can be investigated simply and quickly by assaying for isozymes.

What is electrophoresis?

Electrophoresis is the process by which charged particles move through an electric field. The rate of movement of the particles depends on the magnitude of their charge. Usually, some matrix is required to support the particles and allow the passage of the electric current. These matrices include paper, acrylamide, cellulose acetate, agarose and starch. Electrophoresis can be carried out horizontally or vertically.

Here, we will only consider horizontal starch gel electrophoresis of enzymes. The starch gel has the additional function of acting as a 'molecular sieve'. The gel forms a very regular molecular lattice that impedes the migration of enzyme molecules to a greater or lesser extent, depending on their overall size and three dimensional shape. The separation of enzymes by electrophoresis thus occurs as a result of two processes, (1) the difference in electric charge causing a different rate of movement and (2) difference in size and shape resulting in a different degree of impediment by the molecular sieve.

Uses of isozyme analysis in *Acacia* breeding research

If biochemically suitable material is available, isozyme analysis can be applied to any problem requiring the identification of genetic variants or assessment of levels of genetic variation. Isozyme analysis has been used to address a number of problems in acacias including such topics as:

- validity of interspecific controlled crosses (Wickneswari et al. 1989, Sedgley et al. 1991)
- interspecific hybrid identification (Kiang et al. 1988, Wickneswari 1989, Wickneswari and Norwati, these proceedings)
- species genetic diversity (Moran et al. 1989b)
- genetic variation within and between natural populations (Moran et al. 1989b, Joly and Zeh-Nlo, pers. comm., Playford et al. 1991, Wickneswari and Norwati 1991)
- estimation of outcrossing rates (Moran et al. 1989a).

Studies on genetic diversity of *Acacia* species have shown that provenance identification by geographic zone is possible in some species using isozyme analysis (Joly and Zeh-Nlo, pers. comm., Playford et al. 1991, Wickneswari and Norwati 1991). However, clonal identification using isozyme analysis has not been possible in the *Acacia* species studied so far. For this, techniques which can discriminate individual genotypes as close as possible to the DNA level need to be investigated. Such techniques will include DNA 'fingerprinting' technique utilising VnTR (Variable number Tandem Repeats) probes, RFLP (Restriction endonuclease Fragment Length Polymorphism) analysis utilising nuclear DNA probes with defined base sequences or PCR (Polymerase Chain Reaction). Use of isozyme and DNA markers for detection of economically important traits and disease resistance in acacias (e.g. heartrot and stem borers) needs to be investigated.

Electrophoretic Techniques and Staining Methods

Preparation of plant tissue extracts

The main purpose of this process is to release enzymes from the tissue into an extraction buffer containing various antioxidants and stabilisers that help prevent destruction of the enzyme molecules. Various types of tissue can be used, depending on the objective of the study and the enzymes to be assayed. In this study of acacias, adult leaf and young seedling tissues were used. The young seedling tissues yielded more enzyme systems than the adult leaf tissues.

Adult leaf tissues

Leaf tissue is a very convenient source of enzymes because it is usually abundant and available throughout the year. However, the acacia leaves tend to contain high concentrations of secondary plant metabolites (e.g. tannins and resins) which cause rapid deterioration of the enzymes when the leaves are homogenised. Hence, selection of undamaged leaves and some pretreatment were necessary to maximise the activity of enzymes. In this study, young adult leaves were selected because they contained less breakdown secondary metabolites than older leaves. The leaves were brought to the laboratory, immediately freeze-dried to avoid degradation of enzymes and stored at –20°C until isozyme analysis was carried out.

Approximately 200 mg of freeze-dried leaf segments were placed in a 5 mm diameter mortar. In the case of fresh leaves, 400 mg gave good enzyme activity. A small quantity of liquid nitrogen was then added to the leaves to facilitate grinding and maintain a low temperature so that the enzymes that were released were not denatured. About 0.4 mL of leaf extraction buffer (Appendix I) was added immediately to create a slurry. Care was taken not

to allow the frozen leaf powder to thaw before the extraction buffer was added. The crude extract was filtered through a high strength filter cloth, held in a filter funnel, into an eppendorf tube. The tube with the plant extract was then plunged into crushed ice.

These extracts were used immediately for the isozyme analysis by absorbing onto 5 × 10 mm chromatography paper wicks or stored at –70°C and assayed later.

Young seedling tissues

For young seedlings 4–5 days old, the method of tissue extraction was much simpler because it was relatively free of secondary plant metabolites. Germination was facilitated by nicking the seed coat to allow water absorption. Nicked seeds were placed on moist tissue paper in petri dishes. When the radicle was about 1 cm long, the seed coat was removed and the seedling was crushed in 1–2 drops of seed extraction buffer (Appendix I). The extract was then immediately absorbed onto 3.5 × 10 mm chromatography paper wicks and was ready for electrophoresis. The extract can also be stored at –70°C until required for analysis.

The duration of the plant tissue extraction procedure was kept to a minimum to avoid unnecessary denaturation of the enzymes.

Electrophoresis

Gel buffer systems

Four electrophoretic buffer systems were used in this study — histidine, lithium, morpholine citrate and tris citrate buffers. All these buffers were prepared according to the following recipes.

Histidine (H)

Gel buffer : 0.005M histidine HCl (1.05 g/L) adjusted to pH 8.0 with 10N NaOH

Tray buffer : 0.41M tri-sodium citrate (103.2 g/L) adjusted to pH 8.0 with 0.41M citric acid

Lithium (L)

Gel buffer : 9 parts of 0.065M tris (7.787 g/L) + 0.01M citric acid (monohydrate) (2.10 g/L) and 1 part of tray buffer, pH 8.2

Tray buffer : 0.05M lithium hydroxide (2.10 g/L) 0.19M boric acid (11.75 g/L), pH 8.5

Morpholine citrate (MC)

Gel buffer : 1 in 20 dilution of tray buffer

Tray buffer : 0.04M citric acid (monohydrate) (8.40 g/L) adjusted to pH 6.1 with N-(3-amino-propyl)-morpholine (about 10.5 mL/L)

Tris citrate (TC)

Gel buffer : 0.1M tris (12.1 g/L) 0.0069M citric acid (monohydrate)(1.45 g/L), pH 8.6

Tray buffer : 0.3M boric acid (18.5 g/L) 0.1M NaOH (4.0 g/L), pH 8.6

Different gel buffer systems were used for different enzyme systems. Table 1 shows the enzyme systems that were best resolved in the different gel buffer systems used for acacias. Buffers were prepared and stored at 5°C. Tray buffers were reused once.

Gel preparation

Starch gel was used in this study because many samples (50) could be run and many enzymes (6) could be stained per gel. Ten per cent starch gel was prepared by weighing the required amount (34 g for 340 mL buffer) of starch powder (Sigma soluble potato starch) in a 500 mL conical flask. When making the gel, about two-thirds volume of cold buffer solution was heated to boiling. The remaining buffer solution was poured into the flask containing the starch powder and shaken to create a suspension. The starch suspension was shaken continuously to prevent sedimentation of the starch powder. Then the boiling buffer solution was quickly poured into the starch suspension and shaken vigorously to form a smooth colloid free of lumps. The starch gel was immediately degassed using an aspirator. When large bubbles started to form, the gel was poured immediately into a 23 × 12 × 1 cm gel mould and allowed to set overnight. The starch gel was covered with cling wrap to prevent desiccation.

Loading of samples

Prior to loading, saturated paper wicks of tissue extract were prepared. The cling wrap was folded back lengthwise to expose about 4 mm width of gel. A lengthwise cut was then made about 3 cm from the long edge of the exposed gel. The narrow portion of the gel was the cathodal strip and the wide portion was the anodal strip. The line of cut was the origin.

The two portions of the gel were carefully separated by pushing back the cathodal strip about 1 cm apart from the anodal strip. Using a pair of sharp pointed forceps, one wick saturated with tissue extract was taken from each sample, blotted on tissue paper to remove excess extract and placed vertically on the cut edge of the anodal strip. About 1 to 1.5 mm space was left between each sample wick to prevent lateral contamination and to facilitate scoring of the gels. After loading all sample

Table 1. Details of enzymes stained for, their abbreviations, enzyme commission number, gel buffer systems on which they were scored, staining methods and references for staining.

Enzyme name	Abbreviation	E.C. number	Gel buffer	Staining method	Reference
Aconitate hydratase	Aco	E.C.4.2.1.3	MC	Agar overlay (L&S)*	Yeh and O'Malley 1980
Alcohol dehydrogenase	Adh	E.C.1.1.1.1	L	Agar overlay (L&S)	Tanksley 1979
Aspartate aminotransferase	Aat	E.C.2.6.1.1	L	Staining tray (L&S)	Conkle et al. 1982
Catalase	Cat	E.C.1.11.1.6	TC	Staining tray (L&S)	Moran, pers. comm.
Diaphorase	Dia	E.C.1.6.4.3	H	Agar overlay (S) Staining tray (L)	Brewer et al. 1967
Esterase	Est	E.C.3.1.1.-	L	Staining tray (L&S)	Wickneswari, unpubl.
Glutamate dehydrogenase	Gdh	E.C.1.4.1.2	TC	Agar overlay (S) Staining tray (L)	Hartman et al. 1973
Glycerate dehydrogenase	Gly	E.C.1.1.1.29	TC	Agar overlay (S) Staining tray (L)	Moran, pers. comm.
Glucose phosphate isomerase	Gpi	E.C.5.3.1.9	H	Agar overlay (L&S)	Delorenzo and Ruddle 1969
Isocitrate dehydrogenase	Idh	E.C.1.1.1.42	MC	Agar overlay (S) Staining tray (L)	Fine and Costello 1963
Leucine aminopeptidase	Lap	E.C.3.4.11.1	MC	Staining tray (L&S)	Adams and Joly 1980
Malate dehydrogenase	Mdh	E.C.1.1.1.37	MC	Agar overlay (L&S)	Brown et al. 1978
Malic enzyme	Me	E .C.1.1.1.82	MC	Agar overlay (L&S)	Soltis et al. 1983
Menadione reductase	Mr	E.C.1.6.99.2	H	Staining tray (L&S)	Moran, pers. comm.
Peptidase	Pep	E.C.3.4.13.11	L	Agar overlay (L&S)	Scandalios 1969
Peroxidase	Per	E.C.1.11.1.7	L	Staining tray (L&S)	Brewbaker et al. 1968
Phosphoglucomutase	Pgm	E.C.2.7.5.1	H	Agar overlay (L&S)	Tanksley 1979)
Phosphogluconate dehydrogenase	Pgd	E.C.1.1.1.44	MC	Agar overlay (S) Staining tray (L)	Moran and Hopper 1983
Shikimate dehydrogenase	Sdh	E.C.1.1.1.25	TC	Agar overlay (S) Staining tray (L)	Tanksley and Rick 1980
Succinate dehydrogenase	Sudh	E.C.1.3.99.1	TC	Agar overlay (L&S)	Brewer and Sing 1970
Triosephosphate isomerase	Tpi	E.C.5.3.1.1	H	Agar overlay (L&S)	Soltis et al. 1983
Uridine diphosphogluconate pyrrophosphatase	Ugp	E.C.	TC	Agar overlay (L&S)	Moran, pers. comm.

* L: adult leaf tissue; S: young seedling tissue

wicks, the cathodal strip was firmly pushed back against the anodal strip.

Separation of isozymes

For each buffer system used, about 400 mL of tray buffer was added to each side tank of the tray. The loaded gel was placed on the tray and the cling wrap was folded back to expose 1.0 cm of the gel surface at both ends. Sponge wicks saturated with electrode buffer from the two buffer tanks were then placed on the exposed surfaces of gel and a good contact was ensured with the gel surface by gently pressing the sponge wicks. The cling wrap was then gently pulled over to cover the sponge wicks. A glass plate was placed on the gel to hold the sponge wicks in place. An ice bag was placed on top of the glass plate to prevent surface heating of the gel. Electrophoresis was carried out in a refrigerator at 5°C. Separation of isozymes was carried out at 65 mA constant current for morpholine citrate buffer, 60 mA constant current for lithium and tris citrate buffers and 45 mA constant current for histidine buffer. After the gels were run for 30 minutes, the sample wicks were removed to prevent tailing of bands and provide unimpeded migration of enzymes. Gels were run for 5–6 hours when the extraction buffer front had migrated about 7 cm from the origin.

Gel slicing and staining

When the run was completed, the input and output plugs were disconnected and the gel was removed from the tray. The cling wrap which covered the gel was removed and the portion beyond the extraction buffer front line was cut and discarded. The gel was sliced into 2 mm thick slices with a piece of 2.3 kg fine fishing line using plastic guide strips of 2 mm thickness placed at both the anodal and cathodal ends of the gel.

About 6 slices were produced from each gel. The top slice was unsuitable for staining. The rest of the slices were transferred to staining trays or perspex plates. Each slice was stained for an enzyme system. Enzyme Staining Recipes are given in Appendix II.

Gels were incubated at 37°C for 30–60 minutes or until bands had developed. If the staining tray method was used, it was necessary to wash the gel with a few changes of water before photographs were taken. In the case of the agar overlay method of staining, photographs were taken once the bands had developed. Later, the zymograms were fixed with 5% acetic acid solution. Zymograms were scored and genotypes recorded.

Data Analysis

If the isozyme data required subsequent analysis for population genetics parameters such as allelic frequencies, percentage of polymorphic loci, heterozygosity or genetic similarity, then the computer program, Biosys-1 (Swofford and Selander 1989), was used. However, if the isozyme data were used to estimate breeding system parameters, then MLTF (Ritland and Jain 1981) was used.

Isozymes of Acacias

To date about 42 isozyme loci from 22 enzyme systems have been assayed from *Acacia* species. Table 2 summarises the enzyme systems, the loci controlling each enzyme system and the number of alleles detected.

Table 2. Isozymes of acacias.

Enzyme system	Maximum no. of loci detected	Maximum no. of alleles detected at any one locus
Aconitate hydratase	2	3
Alcohol dehydrogenase	2	2
Aspartate aminotransferase	4	4
Catalase	1	2
Diaphorase	1	3
Esterase	2	3
Glutamate dehydrogenase	1	3
Glycerate dehydrogenase	1	1
Glucose phosphate isomerase	2	4
Isocitrate dehydrogenase	2	3
Leucine aminopeptidase	2	3
Malate dehydrogenase	2	2
Malic enzyme	1	3
Menadione reductase	4	2
Peptidase	2	1
Peroxidase	4	3
Phosphoglucomutase	2	3
Phosphogluconate dehydrogenase	2	2
Shikimate dehydrogenase	1	4
Succinate dehydrogenase	1	1
Triosephosphate isomerase	1	1
Uridine diphosphogluconate pyrrophosphatase	2	4

Some estimates of genetic diversity based on isozyme loci in *Acacia* species are given in Table 3. Generally, the species exhibit moderate to high genetic diversities and would support a selective breeding program.

Conclusions

Isozyme analysis has proved to be a useful tool in acacia tree breeding research. The important areas where isozyme analysis can be used are in determining the genetic structure of natural populations and hybrid verification between species. In areas where isozyme analysis cannot be used, e.g. clonal identification, DNA fingerprinting techniques need to be developed for acacias.

Table 3. Estimates of genetic diversity based on isozyme loci in *Acacia* species.

Species	No. of populations	No. of loci	Percentage of polymorphic loci (0.99 criterion)	Mean expected heterozygosity	Reference
A. albida	11	17	88–100*	–	Joly and Zeh-Nlo, pers. comm.
A. auriculiformis	2	18	–	0.146	Moran et al. 1989
A. auriculiformis	18	12	39.80	0.081	Wickneswari and Norwati 1991a
A. crassicarpa	2	18	–	0.141	Moran et al. 1989a
A. mangium	11	30	12.73	0.017	Moran et al. 1989b
A. melanoxylon	27	30	55.30*	0.179	Playford et al. 1991

* Criterion for percentage of polymorphic loci not stated
– Estimate not given

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Appendix I

Plant Tissue Extraction Buffers

Leaf extraction buffer A

25 mg	Ascorbic acid (sodium salt)
85 mg	Ethylenediaminetetraacetic acid (disodium salt, dihydrate)
190 mg	Na2S2O5
400 mg	Borax
1000 mg	Egg albumin
50 mg	Dithiothreitol
450 mg	Sodium diethyldithiocarbamate
10 mg	Nicotinamide adenine dinucleotide phosphate
20 mg	Nicotinamide adenine diphosphate
1 mg	Pyroxidal-5- phosphate
50 mL	Polyvinyl pyrrolidone(PVP)-sucrose buffer pH 6.8

PVP–sucrose buffer = 100 g sucrose
140 g PVP 40 (40 000 MW)
20 g PVP 360 (360 000 MW)
1000 mL 0.1M phosphate buffer pH 6.8

Reference: Wickneswari (1989)

Leaf Extraction Buffer B

0.05M	Borate buffer pH 8.0
0.01M	Ascorbic acid
0.5M	Sucrose
1%	Tween 80
0.2%	$MgCl_2$
0.2%	$CaCl_2$
1%	20M Polyethylene glycol
1%	Tergitol
0.1%	β-mercaptoethanol
0.5%	2-Phenoxyethanol
0.005M	Ethylenediamine tetraacetic acid (disodium salt, dihydrate)
2%	Egg albumin
0.006M	Dithiothreitol
0.05M	Sodium diethyldithiocarbamate
0.02M	$Na_2S_2O_5$

Note: Leaf extraction buffer B produces better bands and more enzyme systems.

Seedling extraction Buffer

0.05M	Borate buffer pH 9.0
1 mg/mL	Dithiothreitol
20 mg/mL	PVP–40 (MW = 40 000)

Appendix II

Enzyme Staining Recipes

Aconitate hydratase (E.C.4.2.1.3)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 8.0	3.5 mL	
	$MgCl_2.6H_2O$ 10%	500 µL	
	NADP 10 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL	
	PMS 2 mg/mL	280 µL	
	Cis-Aconitate 2% pH 7.5	2.5 mL	
	Isocitrate dehydrogenase (I.U.3.8 units)	5 µL	

Incubate in the dark at 37°C until blue bands appear.

Alcohol dehydrogenase (E.C.1.1.1.1)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 8.0	6 mL	
	NAD 10 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL	
	PMS 2 mg/mL	280 µL	
	Absolute ethanol	500 µL	

Incubate in the dark at 37°C until blue bands appear.

Aspartate aminotransferase (E.C.2.6.1.1)

0.2M Phosphate buffer + 50 mg/mL PVP pH 7.5	5 mL
0.05% Pyroxidal-5-phosphate	0.5 mL
4.56% L-aspartic acid pH 7.5	1 mL
1.6 % alpha-keto-glutaric acid pH 7.5	1 mL
Fast blue BB salt predissolved in 2 mL distilled water	50 mg

Incubate in the dark at 37°C until dark blue bands appear.

Catalase (E.C.1.11.1.6)

Soak gel in 150 mL 0.2M phosphate buffer pH 6.5 for 30 minutes
Soak in 2% KI for 2 minutes
Soak in 0.03% H_2O_2 until white bands appear.

Diaphorase * (E.C.1.6.4.3)

A.	0.1M Tris pH 7.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 7.0	7 mL (50 mL)	
	Phenolindo-2, 6-dichlorophenol 1 mg/mL	500 µL	
	NADH 10 mg/mL	400 µL	
	MTT 10 mg/mL	400 µL (800 µL)	

Incubate in the dark at 37°C until light blue bands appear.

Esterase (E.C.3.1.1.-)

0.1M Phosphate buffer pH 6.5	50 mL
Fast blue RR Salt	50 mg
Alpha-naphthyl acetate	25 mg (dissolved in 2 mL acetone)
Beta-naphthyl acetate	25 mg (dissolved in 2 mL acetone)

Incubate in the dark at 37°C until reddish-black bands appear.

Glutamate dehydrogenase* (E.C.1.4.1.2)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 8.0	6 mL (50 mL)	
	$CaCl_2$ 10 mM	28 µL	
	NAD 10 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL (560 µL)	
	PMS 2 mg/mL	280 µL (560 µL)	
	Sodium glutamate	150 mg	

Incubate in the dark at 37°C until blue bands appear.

Glycerate dehydrogenase * (E.C.1.1.1.29)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B
B.	0.1M Tris pH 8.0	6 mL (50 mL)	
	Glyceric acid	170 mg	
	NAD 10 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL (560 µL)	
	PMS 2 mg/mL	280 µL (560 µL)	

Incubate in the dark at 37°C until blue bands appear.

Glucose phosphate isomerase (E.C.5.3.1.9)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to
	Agar	150 mg	40°C and add to B.
B.	0.1M Tris pH 8.0	6 mL	
	Fructose-6-phosphate 10 mg/mL	1 mL	
	NADP 5 mg/mL	400 µL	
	NBT 10 mg/mL	280 µL	
	PMS 2 mg/mL	280 µL	
	10% $MgCl_2.6H_20$	500 µL	
	Glucose–6-phosphate dehydrogenase (I.U.5000 units)	25 µL	

Incubate in the dark at 37°C until dark blue bands appear.

Isocitrate dehydrogenase * (E.C.1.1.1.42)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B
B.	0.1M Tris pH 8.0	6 mL (50 mL)	
	NADP 5 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL (560 µL)	
	PMS 2 mg/mL	280 µL (560 µL)	
	10% $MgCl_2.6H_20$	1 mL	
	DL-Isocitrate 10 mg/mL	1.4 mL	

Incubate in the dark at 37°C until dark blue bands appear.

Leucine aminopeptidase (E.C.3.4.11.1)

0.1M Tris-maleate pH 5.5	50 mL
Fast Black K salt	25 mg
L-leucyl-beta-naphthylamide HCl (dissolved in 2 mL methanol)	25 mg

Incubate in the dark at 37°C until purple bands appear.

Malate dehydrogenase (E.C.1.1.1.37)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B
B.	0.1M Tris pH 8.0	6 mL	
	NAD 10 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL	
	PMS 2 mg/mL	280 µL	
	1M DL-malate	400 µL	

Incubate in the dark at 37°C until blue bands appear.

Malic enzyme (E.C.1.1.1.82)

A.	0.1M Tris pH 7.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B
B.	0.1M Tris pH 7.0	7 mL	
	NADP 5 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL	
	PMS 2 mg/mL	280 µL	
	10% $MgCl_2.6H_20$	1 mL	
	1M DL-malate	400 µL	

Incubate in the dark at 37°C until blue bands appear.

Menadione reductase (E.C.1.6.99.2)

A.	0.1M Tris pH 7.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B
B.	0.1M Tris pH 7.0	7 mL	
	Menadione	40 mg	
	NADH 10 mg/mL	400 µL	
	NBT 10 mg/mL	400 µL	

Incubate in the dark at 37°C until dark blue bands appear.

Peptidase (E.C.3.4.13.11)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to
	Agar	150 mg	40°C and add to B
B.	0.1M Tris pH 8.0	7 mL	
	L-leucyl alanine	25 mg	
	Crude peroxidase (I.U.5000 units)	1 mg	
	L-amino acid oxidase (I.U.8.9 units)	30 µL	
	3-amino-9-ethyl carbazole	5 mg predissolved in 5 drops methanol.	

Incubate in the dark at 37°C until dark brown bands appear.

Peroxidase (E.C.1.11.1.7)

Absolute ethanol	17 mL
0.2M acetate buffer pH 4.6	7 mL
0-dianisidine	25 mg
3% H_2O_2	500 µL

Incubate in the dark at room temperature until dark brown bands appear.

Phosphoglucomutase (E.C.2.7.5.1)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 8.0	5 mL	
	NADP 5 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL	
	PMS 10 mg/mL	280 µL	
	1M $MgCl_2.6H_2O$	140 µL	
	Glucose-1-phosphate 20 mg/mL	1 mL	
	Glucose-6-phosphate dehydrogenase (I.U.5000 units)	20 µL	

Incubate in the dark at 37°C until dark blue bands appear.

Phosphogluconate dehydrogenase* (E.C.1.1.1.44)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 8.0	5 mL (50 mL)	
	NADP 5 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL (560 µL)	
	PMS 2 mg/mL	280 µL (560 µL)	
	1M $MgCl_2.6H_2$)	1 mL	
	6-Phosphogluconic acid 10 mg/mL	280 µL	

Incubate in the dark at 37°C until blue bands appear.

Shikimate dehydrogenase* (E.C.1.1.1.25)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to 40°C
	Agar	150 mg	and add to B.
B.	0.1M Tris pH 8.0	6 mL	
	NADP 5 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL (560 µL)	
	PMS 2 mg/mL	280 µL (560 µL)	
	Shikimic acid	25 mg	

Incubate in the dark at 37°C until blue bands appear.

Succinate dehydrogenase (E.C.1.3.99.1)

A.	50mM Na phosphate pH 7.0	10 mL	boil and cool to
	Agar	150 mg	40°C and add to B.
B.	50mM Na phosphate pH 7.0	6 mL	
	Ethylenediaminetetraacetic acid	35 mg	
	Adenosine triphosphate (disodium)	7 mg	
	NAD 10 mg/mL	400 µL	
	MTT 10 mg/mL	280 µL	
	PMS 2 mg/mL	280 µL	
	Sodium succinate	35 mg	

Incubate in the dark at 37°C until blue bands appear.

Triosephosphate isomerase (E.C.5.3.1.1)

A.	0.1M Tris pH 8.0	10 mL	boil and cool to
	Agar	150 mg	40°C and add to B.
B.	0.1M Tris pH 8.0	8 mL	
	Sodium pyruvate	50 mg	
	DL-alpha-glycerophosphate	100 mg	
	NAD 10 mg/mL	400 µL	
	Lactate dehydrogenase (I.U.1000 units)	10 µL	
	Alpha-glycerophosphate dehydrogenase (I.U.5000 units)	12 µL	

Incubate at 35°C for one hour
Adjust pH to 3.0 with 10% HCl, then 8.0 with 1.0N NaOH

Add:

Sodium arsenate	10 mg
Ethylenediaminetetraacetic acid	10 mg
NAD 10 mg/mL	400 µL
MTT 10 mg/mL	280 µL
PMS 2 mg/mL	280 µL
Glyceraldehyde-3-phosphate dehydrogenase (I.U.5000 units)	22 µL

Incubate in the dark at 37°C until dark blue bands appear.

Uridine diphosphogluconate pyrrophosphatase

A.	0.1M Tris pH 8.0	17 mL boil and cool to 40°C
	Agar	150 mg and add to B.
B.	0.1M Tris pH 8.0	5.5 mL
	Uridine 5'-diphosphoglucose 25 mg	
	Sodium pyrophosphate	10 mg
	10% $MgCl_2.6H_20$	0.5 mL
	NADP 5 mg/mL	400 µL
	MTT 10 mg/mL	280 µL
	PMS 2 mg/mL	280 µL
	Glucose-1,6-diphosphate 5 mg/mL	500 µL
	Phosphoglucomutase (I.U. 1000 units)	20 µL
	Glucose-6-phosphate dehydrogenase (I.U. 5000 units)	20 µL

Incubate in the dark at 37°C until dark blue bands appear.

Abbreviations used

NADH = Nicotinamide adenine dinucleotide (reduced)
NAD = Nicotinamide adenine dinucleotide
NADP = Nicotinamide adenine dinucleotide phosphate
MTT = (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
PMS = Phenazine methosulfate
NBT = Nitro blue tetrazolium
Tris = Tris (hydroxymethyl) aminomethane
PVP = Polyvinylpyrrolidone

* This enzyme was stained using the staining tray method for leaf tissue. The amount in brackets was used for leaf tissue.

Breeding Hybrids of Forest Trees: Definitions, Theory, Some Practical Examples, and Guidelines on Strategy with Tropical Acacias

D.G. Nikles* and A.R. Griffin†

Abstract

Concepts of interspecific and interprovenance hybridisation of forest trees are described and related to the better-known examples of hybrids of crop plants and domestic animals. The roles of complementarity and heterosis in hybrid vigour are outlined.

Although it is by no means certain that hybrids will be superior to pure populations in one or more environments, there are examples of successful forest tree hybrids. This knowledge encourages further research and development. Guidelines for such work are derived from 'working principles' deduced from results of recent work on natural and manipulated hybrids, including production and testing of forest tree hybrids in Queensland and elsewhere.

There are strong indications that a good knowledge of the characteristics of individual species and populations which may be candidates for hybridisation, and of the environments in which hybrid progeny will be grown, can narrow the choice of likely suitable pairs of populations, i.e. those with complementary traits. It is pointed out, however, that hybrids must be tested against valid controls, and that it is likely that genetic improvement of parental populations will be a useful prelude to regular crossing between populations. Where heterosis makes an important contribution to hybrid superiority, more complex breeding schemes such as reciprocal recurrent selection may be required.

It is concluded that there are likely to be good opportunities for development of useful interspecific and perhaps interprovenance hybrids in tropical *Acacia* species.

SINCE some spontaneous hybrids of *A. mangium* and *A. auriculiformis* appear to be highly productive (Pinso and Nasi, these proceedings), it seems reasonable to assume that even higher quality can be obtained by a systematic approach to hybrid breeding. However this presents a number of significant technical problems and, in practice, hybrids have been used rather sparingly in exotic tree plantation programs (Zobel et al. 1987). Careful analysis is therefore required in deciding whether this is an appropriate means of improving these tropical acacias. In this paper we illustrate approaches to hybrid breeding by reference to a number of successful case studies, and also develop some guidelines appropriate to the acacia situation.

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Definitions

We need to have a clear understanding of what we mean by 'hybrids'. Perhaps the most common perception is that associated with hybrid corn. In this case, hybrids are crosses between highly inbred 'lines'. The lines are derived from selected plants by several generations of self-pollination.

The selfing of corn plants results in a loss of vigour, as it does in many (perhaps all) outcrossing, heterozygous tree species. Vigour is restored, however, on crossing between the inbred lines. Both single crosses (e.g line A × line B) and double crosses [e.g (A × B) × (C × D)] are used for crop production.

The term hybrid is also used for crosses between plants or animals of different populations (either within a species, i.e. provenances or breeds, or of different species). In corn breeding, it has been been found necessary

to use the term 'unconventional hybrids' for crosses between populations or races (Paterniani 1990). Of course, hybrids are developed by crossing individual plants or animals. Clearly there is much room for confusion unless there is definition of (1) the inbreeding status of the individuals used for crossing; and (2) their population status (provenances within a species or provenances from different species).

Many forest tree species exhibit great genetic variation among provenances, so crosses between disparate provenances might involve more genetic difference of the parents than there is for crosses between some species. Therefore it is useful to specify whether hybrids are interprovenance (within species) or interspecific. It is usually considered (but not proved) that crossing between provenances within a species is not accompanied by reduced viability and the kind of advanced generation 'hybrid breakdown' which may occur in interspecific crosses.

As well as understanding hybrids in general, we need to realise that various advanced-generation hybrids can be derived from the first generation (i.e. F_1) crosses; for example:

(a) F_2, F_3, F_4, etc. hybrids, and

(b) Backcross hybrids.

F_2, F_3, F_4, etc. hybrids are derived by successive generations of crossing among hybrids. In such recurrent breeding, inbreeding may be allowed or there may be a conscious effort to minimise the build-up of inbreeding by avoiding the mating of relatives (for as long as possible). This leads to the development of a 'composite' hybrid population. Inbreeding is usually avoided in forest tree breeding because of inbreeding depression of growth and fecundity. In forest trees some out-cross breeding to the F_2 (and perhaps higher) generations has been undertaken, e.g. with some *Pinus* species in Queensland (Nikles et al. 1987).

Backcross hybrids of parental populations A and B are produced by mating A × B F_1 individuals to F_0 individuals of A or B (or both). Backcrossing, usually for a number of generations, is used most extensively in crop plant breeding and some other plants (and animals) where generation interval is short, and there is a need to recover plant or animal 'type' after initial crossing. The backcrossing is usually done to incorporate new genes such as those for disease resistance. Backcrossing for a single generation has been carried out extensively with *Pinus* in Queensland, and several such backcrosses are very promising, for example the *P. elliottii* var. *elliottii* (PEE) × *P. caribaea* var. *hondurensis* (PCH) F_1 hybrid backcrossed to PCH has high seed viability, very good early growth rate and great wind firmness.

We can also develop 'complex', i.e. multi-provenance or multi-species hybrids such as trihybrids, four-species hybrids. Such hybrids are common in animal breeding (Nicholas 1987), and have been produced with *Pinus* in Queensland e.g. PEE × (PCH × *P. tecunumanii*) which has been derived from the vigorous and widely adapted F_1 (Nikles 1989) and PEE.

At another level of consideration, hybrids of various kinds (family structures) can be produced depending on the mating design — just as in crosses made within a population. Thus open-pollinated, polycross, 'haphazard' (non-systematic), nested, factorial, etc., but not diallel, mating designs can be used to develop different kinds of hybrid families.

The terms heterosis and hybrid vigour are not always understood. Some workers consider them synonyms while others use hybrid vigour to refer to hybrid superiority without any causal connotation, and heterosis to refer to superiority due to non-additive gene effects.

Hybrid superiority may be in relation to the mean value of the parents ('mid-parent') or to 'high parent' or 'low parent'. The base should be specified unless it is obvious from the context. When developing hybrids for operational use, performance relative to the high parent is more appropriate.

Theory

Quantitative and Mendelian genetic theory is well developed for hybrids of inbred lines of corn (Hallauer and Miranda 1981). Relatively little, however, is known about genetic parameters in hybrid populations of forest trees or, indeed, of other plant populations (Gallais 1988). Therefore, theory is poorly developed at present. It is better developed in animal genetics and breeding (Falconer 1989). Nicholas (1987) explains that cross-bred progeny of domestic animals usually show heterosis (or hybrid vigour) for certain characters (especially survival and reproductive ability), the amount of heterosis depending on the environment and the genetic diversity of the populations crossed (the greater the diversity, the greater the heterosis — up to a limit). Heterosis may be dependent on non-additive gene action.

Another common feature of cross-breeding in domestic animals is complementarity (Nicholas 1987). This may occur when two or more characters complement each other. Certain crosses show much more complementarity than others depending on the extent to which the populations differ and on the direction of the cross. Complementarity is consistent with additive gene effects.

Thus hybrid superiority may result from heterosis, complementarity, or both.

There is no reason to doubt that in some forest trees, hybridisation may be able to exploit either heterosis or complementarity or both.

What Do We Know about Hybrids of Forest Trees?

The weakness, sterility, and inviability of interspecific hybrids are well known (Stebbins 1958) and are, in fact, a major means by which the integrity of related species is maintained in nature. There is ample evidence, however, that occasional hybridisation and introgressive genetic exchange has played an important part in the evolution of flowering plants. Hybridisation and polyploidy together have been involved in the evolution of several economically important species, most notably the bread wheats. If natural hybridisation has occasionally produced new types that are better fitted to a prevailing environmental niche, then it is reasonable to suggest that artificial hybridisation may also be capable of generating favourable combinations of characters which will be better fitted to mankind's agricultural environments. Hybridisation is a 'numbers game'. For most crosses we must expect many failures before finding a favourable combination of characters that will produce a viable, fertile, and useful hybrid. Intuitively, the failure rate of interspecific crosses in Nature must also be enormously high, but Nature, of course, has a much longer period in which to work. Improved technology will make it possible to generate greater numbers of a wider range of hybrids, and thus increase our chances of success.

This quotation is from a paper by Williams (1987) covering interspecific hybridisation in pasture legumes. In these and many other seed-propagated plants, such as the cereals and grasses, hybrids must be fertile (or capable of having fertility restored) to be useful. But hybrid infertility may not be a 'barrier to commercial exploitation of vegetatively propagated plants such as ornamentals or trees which are readily grown from cuttings' (Williams 1987), or of trees in which a seed-propagated F_1 generation is used as the commercial crop.

In a detailed review of heterozygosity, heterosis, and fitness in outbreeding plants (primarily forest trees), Ledig (1986) concluded that, although capacity for hybridisation is fairly common among forest trees, 'heterosis is not a general phenomenon in interspecific hybrids, nor is it common in wide interpopulation crosses'. He also stated that 'hybridisation of divergent genomes in wild plants rarely increases vigour or fitness' and 'the bulk of evidence suggests that heterosis in both natural and cultivated plant species arises merely from the avoidance of inbreeding'.

In view of these statements about hybrids, it would be wise to take a cautious approach to hybridisation as a method of genetic improvement of forest trees.

Our limited knowledge of the potential of hybridisation in forest tree breeding, of how to choose species and provenances for crossing, and of how to breed the appropriate parental populations for co-improvement, is derived from information on natural hybrids and on the results of studies of manipulated hybrids.

Natural hybrids

Natural interspecific hybrids occur in many plant genera including many forest trees. They are known from *Acacia*, *Eucalyptus*, *Pinus*, *Populus*, *Salix*, etc. In *Eucalyptus*, natural hybridisation is a rather restricted phenomenon (Griffin et al. 1988). Usually forest tree hybrids are fertile and 'hybrid swarms' may comprise a range of advanced generation including backcross types. Under natural conditions, however, hybrid swarms are usually confined to a zone of overlap of the distributions of the parental species (see, e.g., Potts 1990.)

Natural hybrids often occur when species of the same genera are brought together as exotics. This has occurred frequently with *Eucalyptus* and, of course, with *Acacia*. It is not uncommon to observe really outstanding hybrid individuals in such circumstances. Examples include *Acacia mangium* × *A. auriculiformis*, *Eucalyptus torrelliana* × *E. citriodora*, *E. urophylla* × *E. alba* and *E. tereticornis* × *E. grandis*. Such examples strengthen the view that well planned and directed interspecific hybridisation of some forest trees may be a very good investment.

Manipulated hybrids

A few examples of promising or successful hybrids will be given.

A. Hybrids of local (south Queensland) provenances and of local × widely-separated provenances of *Araucaria cunninghamii* produced and tested in Queensland.

Dieters et al. (1989) reported on six-year growth of a study of 'wide crosses' and controls in *A. cunninghamii* planted at Benarkin (27°S) in southeast Queensland. The trial was replicated at two other locations in the same region (Kenilworth and Gallangowan) for which data are now available. The trial sites varied considerably in soils and climate. Four kinds of crosses were included.

1. Within each of two local (southeast Queensland) provenances — many families. These families may be somewhat inbred.
2. Between local provenances — many families. These families would not be inbred.
3. Between local provenances and a Gillies provenance (from near Cairns, 17°S) — four families (wide crosses).
4. Between local provenances and a Papua New Guinea provenance (7°S) — nine families (very wide crosses).

Unfortunately Papua New Guinea and Gillies pure populations were not available for inclusion. However,

both these populations have been established in trials elsewhere in southeast Queensland on several occasions. Observations on these trials suggest that Papua New Guinea material is relatively well adapted for diameter but not height growth or insect resistance in southeast Queensland; and that it grows very well in tropical North Queensland. It is likely to have a longer growing season and less frost hardiness than local provenances in southeast Queensland.

It was observed that frost damage occurred at Kenilworth. At Benarkin the hybrids appeared to flush over a longer period than the non-hybrids. Both these phenomena were scored, and the trees were measured for height and diameter at six years of age.

The principal results of the studies can be summarised as follows:

1. At all three locations the 'wide-cross' and 'very wide-cross' hybrid families were significantly superior in growth to their 'pure breed' half-sibs.
2. The hybrids had a longer growing season at Benarkin than the 'pure breed' families of southeast Queensland provenances.
3. At Benarkin there were positive correlations of family means across all populations for six-year height and volume with percentage of trees flushing over a four-month winter period.
4. At Kenilworth the hybrids were more susceptible to frost damage than local material. The damage was not serious and it did not adversely affect growth or stem quality.
5. There was great variation between and within all families in growth, flushing and frost damage.

Some tentative conclusions can be drawn from these results:

1. Hybrid vigour of growth (in relation to local material) can be additional to any due to release of populations from neighbourhood inbreeding.
2. Hybrid vigour of growth, in relation to that of pure southern populations, was expressed over a considerable range of environments in southeast Queensland.
3. The longer growing season of the hybrids is a possible physiological explanation of the hybrid vigour — and the greater susceptibility to frost damage.
4. The hybrids appear to combine the relative cold hardiness and good stem quality of the southeast Queensland population with the great growth potential of the Papua New Guinea population to give an intermediate expression of these characteristics. Thus hybrid superiority to local material appears to be due, in part at least, to 'complementarity' rather than 'heterosis'.

These results are contrary to those found generally with temperate coniferous species (Ledig 1986). However, the performance of *A. cunninghamii* inter-provenance hybrids is so encouraging that the Queensland Forest Service (QFS) has incorporated the development of hybrids in the breeding strategy (Dieters and Nikles 1991). Advanced breeding materials of south Queensland provenances (from around 26°S) are being crossed with selected materials of Coen (Queensland) provenances (from around 14°S) and Papua New Guinea provenances such as Bulolo (from around 7°S). Superior hybrid families and possibly individuals will be propagated operationally.

B. Hybrids of *Pinus elliottii* var. *elliottii* (PEE) and *P. caribaea* var. *hondurensis* (PCH).

Work in Queensland has been reported by Nikles et al. (1987), Nikles and Robinson (1989) and Nikles (1991) and earlier papers. They described the long experience with PEE, PCH and various PEE × PCH hybrids. The PEE × PCH F_1 hybrid is so productive and superior to both parental species overall in much of southeast Queensland that it has been adopted for operational planting on a large scale (Nikles 1991).

Results of the Queensland work appear to enable the following tentative conclusions.

1. Expression of hybrid vigour of growth is dependent on environment. For example, on very swampy sites the F_1 hybrid is superior in growth to both parents, while on well-drained sites it is superior to PEE only. These expressions of growth (and other) characteristics conform broadly to an additive model of intermediate tolerance of swampy conditions and length of growing season. (PEE is very tolerant of swampy conditions but has a relatively short growing season, whereas PCH is intolerant of poor drainage and has a virtually continuous growth habit.)
2. The F_1 hybrid is intermediate for most morphological traits and some other traits such as needle and cone length, flowering period, latewood content and wood density. For stem straightness and wind firmness it tends to be more like the PEE parent, i.e. better than exactly intermediate.
3. The F_1 hybrid has a wider range of good adaptation than either parent, suggesting its greater heterozygosity gives it greater environmental buffering.
4. Even where the F_1 hybrid is only as vigorous as, or even somewhat less vigorous than, PCH or alternative taxa such as *P. caribaea* var. *bahamensis*, it is preferred for planting because of other superior qualities. For example, in a trial of Queensland-bred F_1 hybrids and other taxa in Argentina, stem quality of the hybrids is greatly superior to parental and other taxa,

but it is not the most vigorous taxon. However, it is superior in overall value (Nikles 1991).

5. The F_2 hybrid displays considerable segregation, as is expected, yet excellent early growth. Viability of F_2 (and backcross) seed is much higher than that of F_1 seed suggesting a purging of lethal genes occurs. Perhaps these facts indicate a rather close affinity of the parental species.
6. The backcross hybrids tend to display intermediacy of characteristics between F_1 and the recurrent parent.
7. F_1 seedlots show a great range of interspecific incompatibility in, for example, numbers of viable seed per cone. This presents problems in breeding and seed-producing programs. These problems are being addressed through research. Preliminary results suggest seed viability can be increased by parent selection and by environmental manipulation.
8. There is great variability in growth and other traits among and within F_1 hybrid families indicating that selection of F_0 parents for crossing, and of F_1 families and individuals for mass production, would be extremely beneficial in an operational program.
9. For traits of high heritability within populations, such as stem straightness and basic density, there tends to be a good correlation of General Combining Abilities (GCAs) and General Hybridising Abilities (GHAs) (see Nikles and Newton (1991) for explanation of these terms). For growth traits these relationships can be quite variable among samples.
10. In view of these variable correlations it seems reasonable to first undertake population improvement of the individual populations being used for hybridisation, either before or during the hybrid breeding phase. Test crossing may also be desirable, especially where correlations of GCA and GHA are low.
11. Another 'principle' derived from the Queensland experience (particularly the intermediacy of most traits) is that, through a good understanding of the morphological and silvicultural characteristics of pure populations across environments, it is possible to choose pairs of populations which are more likely to give useful hybrids. For example, if it is desired to develop a population which will be vigorous and windfirm in plantations in North Queensland, it could be hypothesised that an F_1 hybrid of PCH and *P. caribaea* var. *caribaea* and/or the backcross from the PEE × PCH F_1 to PCH might be suitable in view of particular characteristics of these taxa. These and other crosses have been made and are being tested. Early results confirm the hypothesis.
12. It is likely that hybrid populations could be stabilised and useful 'composites' developed by means of recurrent selection over several generations.

The interim breeding strategy in use in Queensland for co-improvement of PEE and PCH for superior F_1 hybrid development is a modified form of 'reciprocal recurrent selection'. This involves:

1. identification of a subset of the PEE breeding population whose members have high GHA i.e. make good hybrids with PCH.
2. similar identification of PCH trees with high GHA.
3. intercrossing within each subset to advance each breeding population separately.
4. undertaking mass selection within each advanced pure population; then, either undertaking another round of reciprocal testing, or simply applying another round of recurrent selection within each pure breed before again selecting parents for hybridisation.

At each generation of pure breed development, the 'best' individuals are chosen for crossing to produce F_1 hybrid seed for operational purposes. This procedure is analogous to that of 'continual selection (within each of several populations) and regular crossing (between populations)' which is commonly used in domestic animal breeding (Nicholas 1987), except that with pines in Queensland we are undertaking at least one round of reciprocal testing and selection.

In view of the great potential of the PEE × PCH hybrids in Queensland and elsewhere (Nikles 1991), a special study was established to investigate population and population-by-environment parameters in Queensland with the following genetic materials:

Population	No. of parents	Mating design	No. of families
PEE	12	6 × 6 factorial	36
PCH	12	6 × 6 factorial	36
F_1	12 + 12	12 × 12 factorial	144
F_1	6 + 6	6 × 6 factorial	36*
F_2	12 F_1	6 × 6 factorial	36
Backcross to PEE	6 F_1, 6 PEE	6 × 6 factorial	36
Backcross to PCH	6 F_1, 6 PCH	6 × 6 factorial	36
			360

* These families are reciprocal crosses.

The progeny were planted at four locations in Central-Southeast Queensland early in 1987 in replicated designs with 24 or 30 trees per family per location.

The systematic mating design allows us to delve into the genetic control of early height in the various taxa.

Statistical analyses of three-year F_1 family mean heights have not been completed on the data sets but it is clear that:

1. There is considerable variation of GHAs (ranging 4.33–5.06 m for PEE and 4.36–5.06 m for PCH). This means that some parents give much better hybrids than others on average.
2. There are large variations among the 144 F_1 hybrid family means ranging 3.81–5.50 m. Also, we observed that there is great variation among individual trees of all families. So there is a great deal of genetic variation in this F_1 population. All the hybrid trees are not outstanding.
3. The results obtained fit quite well to an additive model of quantitative inheritance of three year height. For example, mid-parent values predict individual family values quite well. Thus crosses involving PEE parents with high GHAs have high values. Likewise, PCH parents with high GHAs give tall families. (Future detailed analysis of later-age data will include statistical tests of GHA and Specific Hybridising (SHA) effects and will look at the relative sizes of these components of the variation.)
4. A point of major importance in relation to developing strategies for hybrid breeding is that the phenotypic correlations of GCA and GHA values are positive and reasonably strong. For the PEE parents they are of the order of 0.70**. (For some unknown reason the correlations are considerably lower for the PCH parents.)

Results given in (4) above mean that the combining abilities of slash pine seed parents assessed by pure slash pine families are good predictors of GHAs. It follows that the best slash pine parents will tend to give the best hybrids, and that population improvement within slash pine has been a very useful prelude to hybridisation. Likewise the highly successful population improvement of PCH for stem straightness and wind firmness already achieved in Queensland is contributing strongly to the excellence of current hybrids.

C. Manipulated hybrids in *Eucalyptus*

Griffin et al. (1988) reported: 'Most records of manipulated hybrids derive from the commercially important subgenus *Symphyomyrtus*. Combinations between geographically isolated species are frequent and successful crosses have been made between species in different sections, although an increased frequency of viability problems was noted in some cases'.

Martin (1989) reviewed experience with hybrids in *Eucalyptus* (and other genera). He outlined the successful operational use of vegetatively propagated interspecific hybrids in Brazil and Congo. In Brazil, the F_1 hybrid *E. urophylla* × *E. grandis* is widely used, both as seedlings from seed produced in open-pollinated seed orchards each comprising a single clone of *E. urophylla* and several pollinator clones of *E. grandis*, and as rooted cuttings from selected F_1 trees. *E. grandis* × *E. pellita* is also a very good combination in Brazil.

In Congo, the F_1 hybrids *E. tereticornis* × *E. grandis* and *E. alba* × *E. urophylla* are dramatically superior to the parental species. It appears that each of these hybrids combines the great growth potential of one parent with environmental tolerance of the other, resulting in superior performance. In Tasmania, studies of freezing resistance of several eucalypts and their F_1 hybrids have shown that additive genetic variation predominates for this trait (Tibbits et al., in press). Similar intermediacy of expression of parental traits in F_1 hybrids is evident for growth rate also. Additional examples of good combinations of traits from different species (eucalypts and others) are given by Sedgley and Griffin (1989).

Martin (1989) considered that production of hybrids with clear superiority to parental species related to a number of ill-understood factors including: (a) genetic affinity of the species, (b) potential growth rate and other specific characteristics of species being considered for crossing, (c) the adaptational characteristics of candidate species, (d) the environment in which the hybrid is to be grown, (e) the provenances used, and (f) hybrid combining ability of individual parent trees of the species concerned. He concluded there is great potential for realising extra productivity through the use of hybrids, where these can be mass propagated successfully, but that much more information was required to enable prediction of likely useful combinations from among the vast numbers which can be conceived.

A consideration of Martin's review, and more recently available information, suggests that reasonable bases for hypothesising likely useful interspecific combinations are (a) look for possible complementarity, (b) seek species with a fairly close taxonomic-genetic affinity, (c) target environments for planting that are intermediate between those in which the parental species are best adapted, and (d) use the 'best' provenances and individuals.

An Approach to Hybrid Breeding of Tropical *Acacia* Species

Using experiences outlined above of the QFS and some other hybridisation programs, it is of interest to consider what would be an appropriate approach to directed hybridisation in tropical *Acacia* species. For example, whether hybrid breeding is appropriate; how to determine which of the numerous pairs of species/populations have the greatest potential in hybrid combination; and what breeding method might apply? Factors to take into account include:

i) it is very difficult to carry out controlled pollination of *Acacia* species;

ii) it is neither logistically nor economically feasible to empirically test all possible pairs of species/ population crosses; and

iii) Many tropical species of *Acacia* are fast growing and can be propagated vegetatively.

It would appear that a simple or fully controlled system of pollination might be combined with operational cloning, once a limited number of known or likely useful interspecies–population combinations are determined. Where artificial cross-pollination is not practical, hybridisation can only be considered for species with overlapping periods of flowering. Obviously such is the case with *A. auriculiformis* and *A. mangium* because natural hybrids occur. Other naturally occurring interspecific hybrids include *A. mangium* × *A. polystachya* and *A. auriculiformis* × *A. leptocarpa* (Pedley, pers. comm.). Thus a primary requirement for strategic planning will be to determine the phenology of tropical species as exotics.

Another important piece of work required is to determine the principal morphological and silvicultural characteristics across environments, and the genetic affinities of candidate species for planting programs. Such information enables a tentative choice of compatible species with complementary characteristics most likely to endow hybrids between them with hybrid vigour and characteristics enabling appropriate environmental matching and desired utilisation potential. A tentative listing of apparent characteristics of several tropical *Acacia* species is given in Table 1. The species listed, with the possible exception of *A. leptocarpa*, are close taxonomically; and their flowering periods overlap to some extent in some localities (Pedley, pers. comm.). This approach to determining the potentially most useful crosses among many, based on known characteristics and genetic affinities, has also been advocated as means for determining the most likely useful crosses to make among populations of buffalo in Asia (Frisch and Vercoe 1991).

On the basis of the characteristics given in Table 1, it is easy to hypothesise that hybridisation of *A. mangium* and *A. leptocarpa*, *A. mangium* and *A. aulacocarpa*, *A. crassicarpa* and *A. aulacocarpa* and some other pairs of species might be successful in giving more rapid genetic improvement than population improvement alone. For genetic improvement of *A. auriculiformis*, it might be very useful to combine the complementary desirable traits of rapid early growth (PNG provenances) with the high frequency of single stems (north Queensland provenances) by interprovenance crossing.

In the case of a well-established hybrid like *A. mangium* × *A. auriculiformis*, there is likely to be opportunity for improvement via co-improvement of the parental species using recurrent selection within species plus regular crossing between species, or a modified form of reciprocal recurrent selection.

There are many options available for population improvement of the tropical species of *Acacia*. Options range from provenance selection plus mass selection to provenance selection plus sophisticated methods such as full-sib recurrent selection. One practical option is recurrent family plus mass selection with population regeneration in an isolated clonal seed orchard.

For species with overlapping flowering and the likelihood of yielding useful hybrids, some hybrid seed could be produced by open pollination in mixed-species stands or orchards. Other options are available. If F_1 hybrid seedlings are easily distinguished, pure breed and hybrid seedlings from each selected parent tree could be tested to determine GCA and GHA values easily and simultaneously.

For species which do not have overlapping flowering periods, controlled crossing would be necessary to produce hybrids. Since this is a time-consuming and costly undertaking, it would be essential to first demonstrate hybrid superiority by means of a limited number of valid tests based on several parents per species.

Guidelines for a Breeding Strategy to Develop *Acacia* Hybrids

Experience in Queensland and elsewhere as briefly outlined above suggests the following guidelines for a breeding strategy to develop *Acacia* hybrids.

1. Determine the product to be developed.
2. Define breeding objectives and selection traits.
3. Determine whether a hybrid breed is required or whether population improvement within a single species is likely to be satisfactory.
4. Supposing it is found that a hybrid seems to be the most likely successful path, then choose two parental species with complementary desirable characteristics in relation to the traits required to satisfy the breeding objectives. If these species have synchronised flowering periods, a hybrid breeding program may be feasible. Recent technological developments, especially as regards controlled hybridisation (Sedgley et al., these proceedings), suggest it is now possible to undertake a hybrid breeding program with non-synchronous species.
5. Develop a breeding-propagation method which integrates intra- and inter-population improvement with development of highly superior materials for propagation populations.
6. Undertake research to develop and test several potentially useful interspecific hybrids and their parental species, including some interprovenance

Table 1. Tentative listing of some apparent characteristics of several tropical *Acacia* species*

Species	Some apparent characteristics
A. aulacocarpa	• Rapid growth in Sabah • Grows to a large size • Good stem form and mostly single-stemmed • Adapted to a wide range of sites • Relatively slow to seed • Straight stems with fine branches • High wood density
A. auriculiformis	• Not adapted to high elevation areas • Tolerates alkaline soils • Papua New Guinea (PNG) provenances of fast growth, but some (not all) multi-stemmed • North Queensland (NQ) provenances of modest growth, higher frequency of single stems • Resistant to borers • Stems rarely straight • Gives natural hybrids with *A. leptocarpa* and *A. mangium*
A. crassicarpa	• Very fast early growth on many sites • More tolerant than *A. mangium* of weed competition • High wood density • Some provenances adapted to alkaline soils • Relatively slow to seed (3rd to 4th year) • Very susceptible to borers and wind effects
A. leptocarpa	• Very rapid early growth • 100% single-stemmed • Does not seed early • Crown/canopy not dense
A. mangium	• Rapid early growth, especially PNG and far NQ provenances • Dense crowns crowd out weeds • Tolerant of fairly poor, acid soils • Often multi-stemmed (varies with provenance) • Flowers and seeds early • Domesticated populations available (may be inbred) • Susceptible to borers • Good wood properties though lower density than several other tropical species • Gives natural hybrids with *A. auriculiformis*, *A. leptocarpa* and *A. polystachya*
A. polystachya	• Grows to a very large tree • Gives natural hybrids with *A. mangium*

* Sim Boon Liang of Sabah Forest Industries Sdn Bhd contributed some of the information used.

crosses within species, to determine superior interspecific hybrids in appropriate environments.

In the specific case of *A. mangium* and *A. auriculiformis*, it will be essential to make a thorough investigation of the relative performance of F_1 hybrids and valid controls of pure *A. mangium* and *A. auriculiformis*. Valid controls would comprise outcrossed, non-inbred progeny of the same seed parents as those which gave the hybrids. Clearly it must first be established that F_1 hybrids of *A. mangium* and *A. auriculiformis* are considerably superior to controls for important economic traits before embarking on an expensive operational program.

Conclusions

It is by no means certain that hybrids will be better than pure populations. However, hybrids of some forest trees have proved to be useful for cultivation operationally. The chances of securing useful artificial hybrids will be increased if careful thought is given to parental characteristics of a group of candidate species or populations, and a pair or pairs of species are chosen for test crossing on the basis of their complementarity of desirable characteristics. This approach uses additive gene effects. Later on, heterosis due to non-additive effects can be investigated.

In the specific case of *A. mangium* and *A. auriculiformis*, well-planned field trials should be established using broadly and comparably based populations of hybrids and the parental species to test for hybrid superiority before hybrid breeding is undertaken operationally. Meanwhile, recurrent selection within the parental species should be undertaken, to develop improved populations of the pure species, so that improved hybrids can be developed in the future if hybrid breeding is indicated as desirable.

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Vegetative Propagation

Multiplication of Families of *Acacia mangium* and *A. auriculiformis* by Cuttings from Young Seedlings

C.Y. Wong* and R.J. Haines†

Abstract

This study suggests that there will be no major impediments to the use of multiplication by cuttings for the mass production of planting stock of superior families of these species. Single node cuttings can be rooted throughout the year at operationally acceptable levels using simple techniques.

High annual multiplication rates (53 and 232 for *A. mangium* and *A. auriculiformis* respectively) are attainable through a simple sequential propagation system. No operationally significant adverse effects of sequential propagation are evident, suggesting that, if necessary by virtue of very limited availability of seed, very high multiplication factors might be achieved by the addition of further propagation cycles.

Operational multiplication by cuttings of superior families is likely to involve little selection for amenability to the propagation procedures, and loss of genetic gain (in economic traits) associated with such selection should be minimal.

An important role of propagation by cuttings in commercial forestry lies in the multiplication, for large-scale planting, of sexually derived material of superior full-sib families. Such vegetative multiplication is particularly applicable where seed of the desired families is limited in quantity due to difficulties in production. This is likely to be the case for full-sib families of *Acacia mangium*, *A. auriculiformis* and their hybrid. Studies reported elsewhere in these proceedings (Sedgley et al.) have led to the development of control pollination procedures which, although very effective, are sufficiently laborious to render impractical the production of seed and seedlings in quantities required for direct use as operational planting stock.

The value of including a cuttings multiplication phase in an operational propagation system is influenced also by:

- The ease with which cuttings can be rooted.

  High rooting rates are desirable — these influence both the economics of propagation and the multiplication rates achieved. It is important also that cuttings can be rooted using techniques which can be applied economically on a large scale.

- Multiplication rates.

  An operational propagation system must include a system for producing the shoots to be used as cuttings — preferably one which maximises the rate of production of shoots of appropriate quality. Multiplication rates are important. For a plantation program of a particular size, there will be an inverse relationship between the vegetative multiplication rate and the amount of seed required per family.

- Genetic variation in responses.

  Selection applied for reproductive traits will result in loss of genetic gain for economic traits (Haines and Woolaston 1991). It is important that such selection be identified, and taken into account when evaluating propagation options.

This article summarises the results of a series of experiments aimed at an appraisal of the operational feasibility of the use of propagation by cuttings for the multiplication of superior families of *A. mangium* and *A. auriculiformis* through examination of the above factors.

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Materials and Methods

Except where particular factors were under test, the general method was to raise seedlings, and then dissect into single node cuttings when these had reached the 7 to 10 node stage (at several weeks of age). Large phyllodes were trimmed at approximately half of their area. Cuttings were given a basal dip in Seradix 3 and then set in coarse sand under 50% shade with intermittent misting provided between 0700 and 1800 hours. A record was kept of nodal origin of cuttings set. At 20–40 days after setting, the number of roots on each cutting was assessed. Appropriate replication and blocking was used in these experiments.

An important objective of this study was the examination of the multiplication rates attainable through sequential propagation. For this experiment, 30 seedlings of each of *A. mangium*, *A. auriculiformis* and a putative hybrid batch were dissected into single node cuttings. Each of these genotypes was carried through five sequential propagation cycles (for each genotype, a sample of the rooted cuttings available at each cycle was dissected into single node cuttings to form the next generation). In each cycle, nodal position was recorded and rooting assessed in the normal manner. Each cycle involved four months from setting of one cycle of cuttings to dissection of rooted cuttings for the next cycle. Under the criteria of the morphological key of Rufelds (1988), almost all of the 'hybrid' seedlings were indistinguishable from *A. mangium*.

Results

Effects on rooting of cuttings

Cutting size. Under appropriate conditions, one, two and three node cuttings of mangium all rooted well. Single node cuttings confer the advantage of higher multiplication rates, and therefore have been used in all subsequent experiments. Experimentation also demonstrated the beneficial effect of trimming to 50% of large phyllodes.

Nodal position. Nodal origin of each cutting was recorded in all experiments. Results presented in Table 1 are typical. Rooting of the first (in particular) and second nodes from the seedling tip is lower than that of other nodes, and the number of rooted cuttings is also lower.

Auxin treatment. Application of an auxin increased both the rooting percentage and the number of roots per rooted cutting. No significant difference was detected among a number of different formulations with respect to rooting percentage (Table 2), but the use of Seradix 3 (0.8% IBA) promoted the highest number of roots per rooted cutting and also resulted in more rapid rooting. This substance was used in subsequent experiments.

Table 1. Effect of nodal position on rooting of *A. mangium* cuttings

Node	Rooting percentage	Root no. per rooted cutting
1	34	2.3
2	68	2.3
3	85	3.0
4	86	2.9
5	79	2.7

Table 2. Effect of auxin treatment on rooting of *A. mangium* cuttings

Treatment	Rooting percentage	Root no. per rooted cutting
IBA 2000	79	2.8
Seradix 1	73	2.6
Seradix 2	72	2.7
Seradix 3	72	3.9
Trihormone	71	2.2
Control	54	1.6

Results have been presented here for *A. mangium* only. Some experimentation was conducted also for *A. auriculiformis*, which in general rooted more readily and displayed less variation with treatment.

Multiplication rates

Rooting percentages and mean root number per rooted cutting for the five propagation cycles of the seedling multiplication rate experiment are presented in Table 3. Data are based on all cuttings — including the first and second nodes. The multiplication factor at each cycle (cuttings available × rooting percentage) has also been calculated. The multiplication factors presented in Table 3 do not display these mathematical relationships exactly, as they are the means of factors calculated independently for individual seedling genotypes.

The effects of sequential propagation are unavoidably confounded with some variation in environmental conditions during the different cycles. Nevertheless, the results offer no evidence of decline in either rooting percentage or multiplication factor with sequential propagation for either species. Similarly, there is no evidence of a progressive decline in the number of roots per rooted cutting for *A. auriculiformis*. The decline from 5.9 to approximately 3 roots per rooted cutting from propagation cycles one to three in *A. mangium*, on the other hand, may be a significant treatment effect.

Variation in multiplication rate with genotype is illustrated in Table 4.

Data for the 'hybrid' seedlings have not been presented here. In general though, the results are very similar to

Table 3. Rooting, root number and multiplication rate through sequential propagation

Parameter		*A. mangium*	*A. auriculiformis*
Rooting percentage	First cycle	51	90
	Second cycle	63	73
	Third cycle	75	92
	Fourth cycle	60	88
	Fifth cycle	77	76
Root number	First cycle	5.9	13.5
	Second cycle	4.4	14.4
	Third cycle	3.4	7.7
	Fourth cycle	2.9	11.0
	Fifth cycle	3.3	8.7
Multiplication factor	First cycle	3.6	6.3
	Second cycle	2.6	4.9
	Third cycle	4.3	6.0
	Fourth cycle	3.9	8.0
	Fifth cycle	3.0	5.6
Multiplication rate over 5 cycles		1039	9411
Annual multiplication rate		53.3	232.4

Table 4. Multiplication rate frequency distributions for *A. mangium* and *A. auriculiformis*

Multiplication rate	Percentage of seedlings	
	A. mangium	*A. auriculiformis*
0–5	17	3
5–20	10	
20–50	33	
50–100	30	
100–150	7	10
150–200	3	23
200–300		47
300–400		14
400–500		3

those for *A. mangium*, supporting the morphological evidence that these are mainly *A. mangium* seedlings.

Discussion

These results demonstrate that single node cuttings from young seedlings of both *A. mangium* and *A. auriculiformis* can be rooted at operationally very acceptable levels using simple techniques. Cuttings planting stock can be produced rapidly throughout the year. The cost of rooted cuttings is not likely to be substantially greater than that of seedlings produced by current nursery procedures. Production costs may even favour cuttings when the cost of producing seed of high genetic quality is taken into account.

The results of this project have demonstrated that, through sequential propagation, high annual multiplication rates are attainable using very simple technology. The annual multiplication rates achieved (53 and 232 for *A. mangium* and *auriculiformis* respectively) are well in excess of multiplication rates which are being exploited commercially for the mass propagation of superior families of *Pinus* taxa.

The experiments have revealed no evidence of adverse effects of sequential propagation through five cycles — apart from a possible reduction in root number per rooted cutting between cycles one and three in *A. mangium*. Even if a genuine treatment effect, evidence gathered for tropical *Pinus* taxa suggests that a reduction in mean root number to this level is unlikely in itself to constitute a reduction in root system quality. The results thus do not preclude the addition of further propagation cycles, should even higher multiplication factors be required. The multiplication factor required will be governed by the ease with which seed of the desired families can be produced.

As expected, results suggest that genotypes vary with respect to vegetative multiplication rates attainable. Distributions of multiplication rates are such, however, that only a small proportion of genotypes display multiplication rates below levels which would be commercially useful for family forestry. Application of propagation by cuttings to the mass production of superior families of these species is likely therefore to involve little selection for vegetative multiplication rate. Loss of genetic gain associated with such selection is therefore likely to be very small.

Although both species are amenable to multiplication by cuttings, *A. auriculiformis* is clearly the more so. The substantially higher multiplication rates achieved with this species are related to both higher rooting and faster shoot growth. The apparent similarity, in this study, of the 'hybrid' to *A. mangium* with respect to multiplication rates is considered more likely to be due to incorrect classification of the seedlings than to large non-additive effects.

Acknowledgments

Field and nursery work was conducted with the assistance of Mr Hadling Mooching and Mr Edward Chia assisted with supervision of some of the experiments. The commitment of the management of Sabah Softwoods Sdn Bhd to this project is acknowledged.

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Prospects for the Mass Propagation of Superior Selection-Age Phenotypes of *Acacia mangium* and *Acacia auriculiformis*

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Abstract

For *A. mangium*, rooting even from 26-month-old ortets is below that of cuttings from young nursery seedlings, although there is no clear evidence of further decline with ortet age. Similarly, rooting of cuttings from 68-month-old ortets of *A. auriculiformis* is below that of cuttings from young seedlings. The study provided no evidence that rooting levels from field ortets of either species can be improved by using shoots stimulated to develop at a low level on the ortet, or by sequential propagation.

A small proportion of genotypes demonstrated rooting rates similar to those achieved with cuttings from young seedlings. Assuming good growth rates in the nursery, then it appears that up to 10% of genotypes will display annual multiplication rates which are similar to the mean multiplication rates calculated for young seedlings.

Assuming that field performance is satisfactory, phenotypic selection followed by simultaneous multiplication and clonal testing, and then operational planting of the superior genotypes, could be feasible, but would involve selection for rooting. This level of selection for rooting is not likely to be prohibitive, provided that rooting and economic traits are not correlated.

ALTHOUGH substantial gains in plantation productivity are offered by the propagation of superior full-sib families, even greater gains are available through the mass propagation of superior individuals within those families. A common obstacle to the commercial propagation of selected phenotypes of forest plantation species is the decline in both the rooting potential and subsequent growth rate of cuttings with increasing age and height of the ortet. A useful approach, commercially exploited for some species of *Eucalyptus* and showing promise also for tropical *Pinus* taxa, has been the use of cuttings harvested from shoots stimulated to develop on the ortet by decapitation. The response of such shoots is frequently related to the level at which they are stimulated on the stem of the ortet. This approach warrants trial also for *A. mangium* and *A. auriculiformis*.

One impediment to the meaningful comparison of the responses of cuttings from coppice shoots with those from young seedlings is the possibility that the responses of the former are influenced (e.g. rooting is depressed) by physiological factors related to the environment in which the ortet is growing. It is therefore important that studies of the responses of coppice shoots include also the investigation of the responses of second generation cuttings harvested from first generation stool plant cuttings raised and maintained under more controlled, uniform conditions. It is possible furthermore that such sequential propagation may result in an improvement in response, either through selection (within genotype) for more responsive material, or through a direct rejuvenating effect.

This project was designed to examine the influence of ortet age and decapitation treatment on coppicing of field ortets and the subsequent rooting of first and second generation cuttings of *A. mangium* and *A. auriculiformis*, thereby facilitating an evaluation of the prospects for mass propagation of superior phenotypes of these species.

Materials and Methods

The project comprised several experiments examining the effects of pruning and decapitation treatments on the production and rooting of coppice in *A. mangium* trees of varying ages, and some work also on *A. auriculiformis*, for which experimental material was much more limited. Data presented here, selected from two of the experi-

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ments, are representative of the trends observed. One experiment comprised a detailed study for *A. mangium*, while the other included both *A. mangium* and *A. auriculiformis*. From the second experiment, data for the latter species only are presented here.

For *A. mangium*, treatments were applied to trees aged 26, 68 and 92 months. Trees aged 68 months were the only ortets available for *A. auriculiformis*. In each age class, trees were decapitated at either 30, 150 or 320 cm for *A. mangium*, and either 30 or 320 cm for *A. auriculiformis*. Remaining branches were pruned back to short stubs. Treatments were applied to 40 trees per decapitation height per age class for *A. mangium*, and 25 for *A. auriculiformis*. In each age class for *A. mangium*, trees were allocated to 10 blocks each of 12 trees. Each decapitation height treatment was then applied to four trees (selected at random) per block. Blocking was applied also in the experiment involving *A. auriculiformis*.

Stumps were subsequently assessed periodically for numbers of living coppice sprouts. At approximately 80 days after decapitation of the *A. mangium* experiment, (and at 114 days for the experiment incorporating *A. auriculiformis*), two shoots were collected from each of the treated trees (preferentially from the top of the decapitated stump). Shoots were dissected into single node cuttings and set in coarse sand in a greenhouse, following a basal dip in Seradix 3. Nodes 1 to 5 were set from each shoot, and a record kept of node of origin. Intermittent misting was applied during daylight hours.

Twenty-one days after setting, the number of roots on each cutting was assessed, and rooted cuttings were transplanted into a soil medium in containers.

Two cuttings from each genotype (selected to represent about the average stage of development for cuttings available of that genotype) were grown on to provide second generation cuttings. At four months after setting, these first generation cuttings were dissected into single node second generation cuttings, and set as described above. Included as controls were both first and second generation cuttings originating from young seedlings. In both cases, donor stool plants were of similar size to those of coppice origin, and raised in the same environment. The second generation phase of the *A. mangium* experiment was laid out in the greenhouse as two large blocks, each with 10 sub-blocks. Each block comprised cuttings from one first generation ramet of each of the genotypes of coppice origin, and first and second generation cuttings from young seedlings. Allocation of coppice genotypes to sub-blocks followed the field design, each sub-block including genotypes from one field block in each of the age classes.

Rooting was assessed after 21 days in the same manner as for first generation cuttings, and rooted cuttings were transplanted into containers to be grown on for an experiment examining the influence of the treatments on field performance.

Results

Data for the coppicing of field ortets and subsequent rooting of first and second generation cuttings of *A. mangium* are presented in Table 1.

In all age classes, most stems decapitated at 150 or 320 cm produced coppice shoots. Most of the 26 month trees decapitated at 30 cm produced coppice also. For the older trees, however, less than half of the trees decapitated at

Table 1. Coppicing and subsequent rooting of first and second generation cuttings of *A. mangium*.

Ortet age (months)	Decapitation height (cm)	Percentage of stems coppicing	Mean no. coppice shoots	First generation		Second generation	
				rooting percentage	root no.	rooting percentage	root no.
26	30	88	8.4	49	6.4	53	4.5
	150	98	30.3	66	8.5	47	2.9
	320	100	42.9	56	7.5	39	3.2
68	30	45	3.5	54	8.1	29	3.3
	150	95	31.3	39	6.3	22	2.9
	320	98	27.9	43	6.0	10	2.6
92	30	42	1.2	42	5.9	72	3.6
	150	95	12.4	39	8.3	45	3.6
	320	100	15.9	37	6.3	50	4.0
Cuttings ex. young seedlings		77	6.3				
2nd gen cuttings ex. young seedlings		83	5.3				

30 cm responded. In all treatments, many of the shoots produced bore pinnate leaves, particularly at the earlier nodes. These morphologically juvenile shoots were produced at levels in the crown well above those at which pinnate foliage appears on intact seedlings.

No clear and consistent trends in rooting were apparent with age or height of decapitation in the material derived from field ortets. The major difference in the data seems to be that material originating from field ortets displays lower rooting than that from young nursery seedlings. There was no evidence of improvement in rooting of this material from the first to the second generation. Mean number of roots per rooted cutting declined from the first to the second generation.

In the absence of marked variation with ortet age and treatment, data have been pooled to derive clonal frequency distributions for rooting in the second generation of cuttings derived from field ortets (Table 2). Included (in the lowest class) are those clones not represented in the second generation due to zero rooting in the first generation.

Table 2. Frequency distribution for mean clonal rooting in second generation.

Percentage of population	Rooting precentage
0–10	37
10–20	9
20–30	11
30–40	9
40–50	11
50–60	6
60–70	5
70–80	7
80–90	4
90–100	1

These results suggest that a small proportion of ortets yields cuttings which display quite good rooting.

Data for the rooting of *A. auriculiformis* in the first and second generations are presented in Table 3. The results are similar to those for *A. mangium* in that, overall, no improvement in rooting in the second generation is apparent, and rooting is still below that of cuttings harvested from young seedlings. Once again, though, there are some ortets which have yielded high levels of rooting.

Table 3. Rooting of first and second generation cuttings from *A. auriculiformis* aged 68 months.

Decapitation height (cm)	Rooting percentage	
	First generation	Second generation
30	65	64
320	53	57
Cuttings ex young seedlings	88	

It should be pointed out that all rooting percentages presented here are based on settings which included the poorer rooting first and second nodes. Data from other experiments with *A. mangium* in this series display trends consistent with those observed here.

Discussion

The results demonstrate that the development of coppice shoots can be stimulated on most trees, provided that the level of decapitation is not too low. The frequently juvenile morphological characteristics of such shoots are suggestive of a rejuvenation effect of the treatment. For *A. mangium*, however, rooting even from 26-month-old ortets is below that of cuttings from young nursery seedlings, although there is no clear evidence of further decline with ortet age. Similarly, rooting of cuttings from 68-month-old ortets of *A. auriculiformis* is below that of cuttings from young seedlings. The study provided no evidence that rooting levels from field ortets of either species can be improved by using shoots stimulated to develop at a low level on the ortet, or by sequential propagation.

Although these experiments were replicated, clonal means for rooting are based on a relatively low number of cuttings and are therefore subject to error. Nevertheless, it appears that a small proportion of genotypes (perhaps up to 10%) could demonstrate rooting rates similar to those achieved with cuttings, from young seedlings. Assuming that growth rates in the nursery are similar to those of seedlings, then perhaps up to 10% of genotypes will display annual multiplication rates which are similar to the mean multiplication rates calculated for seedlings (approximately 50 and 230 for *A. mangium* and *A. auriculiformis* respectively).

To consider the implications of these multiplication rates for clonal forestry : for a hypothetical annual planting program of 2000 ha made up of 20 clones, perhaps 100 000 cuttings of each clone would be required. At the multiplication rates indicated above, it would be possible to produce this number of cuttings of a genotype in less than three years by sequential propagation from stems producing at least one coppice shoot, for both *A. mangium* and *A. auriculiformis*.

To realise the greatest gains through clonal forestry, it will be necessary to base selection on clonal testing. For relatively short rotations of around seven years, it might be possible to make selections in clonal tests at age three. The time required for a clonal test would thus be sufficient for the production of the operationally required numbers of cuttings in a simultaneous multiplication

phase — for those clones displaying good rooting levels. This assumes that the favourable response to sequential propagation reported elsewhere in these proceedings (Wong and Haines) would continue through several further cycles.

Assuming good nursery and field growth rates, phenotypic selection followed by simultaneous multiplication and clonal testing could thus be feasible, but would involve some selection for rooting — perhaps 1 in 10 for *A. mangium*. Recent calculations (Haines and Woolaston 1991) have shown that the effect, on the capture of genetic gain, of selection at this level for reproductive response need not be prohibitive provided that:

- the intensity of selection for economic traits is high,
- economic and reproductive traits are not adversely correlated.

The extent to which economic and reproductive traits are correlated in *A. mangium* and *A. auriculiformis* remains to be determined in the field trials. The application of suitably intense selection for economic traits should not be difficult. Phenotypic selection is sufficiently inexpensive to permit screening of a large population, and poor rooters could subsequently be culled, prior to the commencement of the more expensive clonal testing phase.

A small decline in rooting with ortet age might be operationally tolerable. Provided multiplication rates were sufficient to provide the required number of cuttings in the time available, the main effect would be a minor increase in the cost of planting stock, which is anyway only a small component of plantation establishment costs. On the other hand, any loss of field vigour of cuttings associated with ortet age is much less likely to be acceptable.

These calculations demonstrate also the feasibility (given the above assumptions) of simultaneous multiplication and clonal testing of juvenile genotypes, using material from young nursery seedlings. With this approach, a much lower intensity of selection for rooting would be imposed than for the coppicing and multiplication of older field ortets, although the gain offered by the phenotypic selection would not be available.

Acknowledgments

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Micropropagation Techniques for *Acacia mangium* × *Acacia auriculiformis*

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Abstract

Plantlets of *Acacia* hybrid were successfully produced from nodal cultures of aseptically germinated seedlings. For shoot formation, the combinations of Murashige and Skoog's medium with various concentration levels of 6-benzylamino purine (BAP) were used. The maximum number of shoots per explant was obtained from cultures containing MS medium supplemented with 3% (v/v) sucrose, 0.6 % (v/v) bacteriological agar and 0.5 mg/L BAP. These adventitious shoots were continuously mass-produced by recycled cultures. For root formation, the excised shoots were first treated with a hormone rooting powder before they were planted into rooting chambers containing unsterilised sand. The rooted propagules showed normal growth and their average heights at 6 and 12 months after potting were 58.5 cm and 109.4 cm respectively.

Acacia mangium Willd × *A. auriculiformis* A Cunn. ex Benth hybrids were first spotted at Ulu Kukut, Sabah in 1971 (Rufelds 1987). In Peninsular Malaysia, hybrid trees were first observed at Compartment 2.4 B Ulu Sedili Forest Plantation in 1989 (Darus and Ab. Rasip 1989). Generally, they have superior characteristics compared with their parents, for example, better stem form and longer clear bole than *A. auriculiformis*, lighter branching with predominant and dominant crowns, circular trunks and smoother bark compared with *A. mangium*. In addition, Mohd. Sukari and Darus (1991) have suggested that the strength properties of timbers from four-year-old hybrids are much better than those of timbers of six-year-old *A. mangium* trees.

I believe that hybrid trees can be used for future plantations. However, due to the difficulty of getting viable seeds from naturally grown hybrids, the development of reliable vegetative propagation techniques, particularly through tissue culture for mass production of improved planting materials, will be of great value.

In this paper, a technique to micropropagate *A. mangium* × *A. auriculiformis* hybrids, and their growth performance after potting in the nursery, are highlighted.

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Materials and Methods

Aseptically germinated seedlings

Hybrid seeds obtained from candidate plus trees at Ulu Kukut, Sabah, were pretreated with hot water (80–90°C) to break dormancy before being surface-sterilised with 45% (v/v) Clorox and 0.1% (v/v) Tween 20 for 20 minutes. After sterilisation, they were cultured in sterilised flasks containing half-strength MS basal medium (Murashige and Skoog 1962) supplemented with 3% sucrose (w/v) and 0.6 % bacteriological agar (w/v). Each flask normally contained about 10 to 20 seeds.

Shoot multiplication and elongation

Nodal explants were excised from one-month-old aseptically germinated seedlings. Each seedling was cut into four nodal segments and one shoot tip. Immediately after cutting, they were cultured in sterilised flasks containing full strength MS basal medium supplemented with 3% sucrose (w/v), 0.6 % bacteriological agar and different concentrations, 0.5 , 1.0, 2.0 and 4.0 mg/L of 6-benzylamino purine (BAP). Ten explants were used for every BAP concentration level. They were incubated in a tissue culture growth room, at a room temperature of about 20±5°C and with a photoperiod of 18 hours using

fluorescent tubes. At one month, all cultured explants were transferred onto a fresh medium which contained the same BAP concentration levels. Observations on the number of shoots produced per explant and the length of developed shoots were carried out on two-month-old cultures.

To study the effect of subsequent recycled cultures on shoot multiplication and elongation, explants were excised from the developed shoots of the previous cultures. These shoots were cut to a smaller size (about 0.2 cm long) and subcultured onto a fresh MS basal medium supplemented with 0.5 mg/L of BAP which was found to be the best combination for shoot multiplication and elongation of explants taken from aseptically germinated seedlings. These studies were recycled for seven passages of 2 months each. Observations were carried out at every passage and the heights of the developed shoots were recorded.

Rooting of excised shoots

All shoots more than 0.5 cm long were used for a rooting experiment. They were treated with Seradix 3 before planting in a misted rooting chamber containing 100% unsterilised river sand. Observations were carried out a month after planting and all rooted shoots, non-rooted shoots and dead shoots were counted. A shoot was considered rooted when a newly developed root was clearly visible (at least 0.2 cm in length). Shoots were considered dead if they had turned brown.

A similar rooting experiment was also conducted for every recycled culture to determine the effect of recycled cultures on rooting percentages.

Results

Shoot multiplication and elongation

All explants started to produce adventitious shoots at two weeks after inoculation. Of the five BAP concentration levels tested, cultures with 0.5 mg/L BAP were found to be the most effective medium for inducing multiple shoot formation. The average number of shoots per explant obtained on MS basal medium supplemented with 0.5 mg/L BAP was 21.6 (Table 1). On the other hand, explants in media supplemented with higher BAP concentration levels produced fewer shoots per explant.

The average length of developed shoots in cultures which contained 0.5 to 1.0 mg/L BAP was more than 0.5 cm. The length of developed shoots in media containing higher BAP concentration levels viz. 2.0 and 4.0 mg/L, was less than 0.5 cm (Table 1).

Table 1. Shoot multiplication of *Acacia* hybrids under different 6-benzylamino purine concentration levels.

BAP concentration (mg/L)	No. of shoots/explant ±SE	Average shoot length (cm) ±SE*
0.5	21.6 ± 3.4	0.61 ± 0.10
1.0	15.6 ± 4.4	0.56 ± 0.07
2.0	10.2 ± 3.0	0.44 ± 0.05
4.0	11.4 ± 3.5	0.41 ± 0.03

* SE = Standard error

Effect of recycled culture on shoot multiplication and elongation

The average number of shoots per explant, and length of developed shoots, increased with increasing culture cycle. In the first culture, where explants were obtained directly from aseptically germinated seedlings, the average number of shoots produced was 21.6 with an average length of 0.61 cm. In the seventh recycled culture, 14 months after initiation, almost all cultured explants induced more shoots with an average of 31.0 shoots per explant and an average shoot length was 1.3 cm (Table 2).

Table 2. Effect of recycled cultures on shoot multiplication and elongation.

Culture cycles	No. of shoots per explant ±SE	Average shoot length (cm) ±SE*
1	21.6 ± 3.4	0.61 ± 0.10
2	n.a.	n.a.
3	21.1 ± 3.4	0.69 ± 0.13
4	26.4 ± 8.5	0.82 ± 0.11
5	25.7 ± 7.0	0.80 ± 0.15
6	30.2 ± 5.9	0.82 ± 0.30
7	33.1 ± 9.5	1.30 ± 0.31

* SE = Standard error, n.a.= not available

Rooting percentage of excised shoots

Excised shoots were rooted easily under in vivo conditions. Shoots from the first culture gave 60% rooting percentage at one month after planting (Table 3). The rooting percentage of excised shoots from subsequent cultures (up to the fourth culture cycle) was greater than the rooting percentage of shoots of the first culture. The rooting percentage of excised shoots from the fifth, sixth and seventh recycled cultures declined to 51%, 50% and 48% respectively (Table 3).

Growth performance

Table 4 shows the cumulative height of potted hybrid propagules over a one-year period.

Table 3. Effect of recycled cultures on rooting percentage

Culture cycle	Percentage rooting ± SE*
1	60.0 ± 10.5
2	n.a.
3	73.0 ± 14.9
4	66.0 ± 12.6
5	51.0 ± 8.7
6	50.0 ± 12.5
7	48.0 ± 6.3

* SE = Standard error

Table 4. Cumulative height (cm) of potted hybrid propagules.

Age (months)	0	2	4	6	8	12
Height (cm)	4.2	7.3	25.6	58.5	91.0	109.4

Discussion and Conclusions

The results showed that MS basal medium supplemented with 0.5 mg/L BAP was the most effective combination for shoot multiplication and elongation of *Acacia* hybrid nodal cultures. It is reported that a lower concentration level of BAP was also good for shoot multiplication and elongation of nodal cultures of aseptically germinated *A. mangium* seedlings (Darus 1988). Similar results have also been observed in the micropropagation study of *A. auriculiformis* presently being carried out. It has also been the author's experience that the MS basal medium, supplemented with a lower concentration of BAP, is effective in inducing shoot multiplication as well as elongation of newly developed shoots of *Acacia* species.

Although it was reported that continuous cultures of some plant species, for example *Actinidia chinensis* (Standardi 1982) and *Acacia mangium* (Darus 1989b) decreased in shoot multiplication as well as shoot elongation, adventitious shoots of *Acacia* hybrid continuously regenerated over 14 months without losing their shoot forming ability and average number of shoots per explant. This phenomenon, in fact has been observed on other cultures as well, for example shoot tip cultures of *Bougainvillea glabra* (Sharma et al. 1981) and *Eucalyptus* spp. (Gupta et al. 1981 and Mehra-Palta 1982).

Excised shoots of *Acacia* hybrid can be easily rooted in rooting chambers containing 100% unsterilised river sand. In general, the rooting percentages of hybrid shoots from the first culture, or even from the subsequent recycled cultures, were less than the rooting percentage of excised shoots of *A. mangium* nodal cultures (Darus 1988). This may possibly be due to 1) different types of rooting media being used and 2) the experiments being carried out at places with different environmental conditions. The results also showed that the rooting ability of the hybrid shoots declined with increasing culture cycles. A similar trend has been reported in *A. mangium* nodal cultures (Darus 1988). The decline in rooting ability of excised shoots from continuous recycled cultures might be due to the ageing effect of the shoots in the cultures.

In terms of growth performance, rooted hybrids potted in 15 × 23 cm polybags containing a 100% forest topsoil were ready for field planting at about five to six months after potting. Their growth rates were good and even faster than potted germinated seedlings and potted rooted stem cuttings (Darus 1989a).

In conclusion, based on the above results, *Acacia* hybrids can be propagated through tissue culture. This technique can be utilised for large-scale planting stock production of genetically selected materials. However, more studies, especially on growth performance, are required before deciding to use this technique for mass production because there are cases where planted propagules tend to produce multiple leaders and other abnormalities after planting in the field.

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Propagation Options for *Acacia mangium*, *Acacia auriculiformis* and their Hybrid

R.J. Haines* and A.R. Griffin†

Abstract

This analysis compares a number of propagation options for these taxa in terms of the genetic quality of the planting stock being used for the establishment of production plantations at a particular time. Components considered are the selection for economic traits facilitated, the effects of selection for reproductive traits, and the time required for identification of the superior families or clones and multiplication of the planting stock.

The use of multiplication by cuttings offers substantial advantages for the mass propagation of superior families. This advantage arises through both a shorter multiplication interval and reduction of the selection pressure for reproductive response.

Clonal forestry offers greater gains, depending on the approach adopted. The propagation of individuals selected purely on phenotype is not an approach which can be recommended — family options can offer greater gains. Realisation of the potential gains through clonal forestry will be dependent on more effective selection — either through the inclusion of family information in a selection index, or preferably the use of data from a clonal test. An attractive option for these *Acacia* species is the mass propagation of clones selected in a test comprising cuttings from young seedlings of advanced generation families. In this case, multiplication for plantation establishment would be conducted simultaneously with clonal testing. For these taxa then, maintenance of juvenility, as applied in this approach, is more promising than rejuvenation — selection for favourable response is less intense, and transfer of gains from the breeding to the production population is more rapid.

Sequential propagation by cuttings provides adequate multiplication rates for the mass propagation of both superior families and tested clones. Higher multiplication rates offer no advantage, and no immediate requirement for micropropagation in an operational propagation system is perceived.

THE optimisation of plantantion productivity requires the use of a propagation strategy which efficiently captures, in the production population, the genetic gains made through breeding. The use of bulked progeny of a rogued open-pollinated clonal seed orchard has been the traditional approach to the capture of such gains. In recent years, however, it has been widely accepted that there are other propagation strategies which offer the potential for more effective capture of genetic gain. These include:

- Restriction of seed collection to the very best open-pollinated half-sib families from orchards of the above type.
- The use of superior full-sib families — propagated by cuttings in some cases, and directly from seed mass produced in specialised orchards in others. This applies in particular to some pine species.
- The use of cuttings of superior individual clones, e.g. of poplars and some eucalypts.

While the above comprise the major approaches currently used operationally, there are other technologies which are being investigated for forest species and which are in commercial use for some other crops, e.g. micropropagation.

Propagation options to be considered are of course governed by biological limitations. These *Acacia* species can be control-pollinated, although this is quite laborious, and demonstrate a significant degree of self-compatibility (Sedgley et al., these proceedings). They can be propagated easily, with high multiplication rates, from juvenile

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cuttings (Wong and Haines, these proceedings) and can be multiplied in vitro (Darus, these proceedings). A small proportion of older trees can be multiplied readily by cuttings (Haines et al., these proceedings). *A. auriculiformis* produces seed rapidly after vegetative propagation from mature material (Griffin et al., these proceedings).

This article is intended to be an analysis of propagation alternatives available for *A. mangium*, *A. auriculiformis* and the hybrid. Major objectives of the analysis are the identification of parameters that are important, and the comparison of some options.

Comparing Propagation Strategies

The method used compares propagation strategies in terms of the genetic quality of the planting stock being established in production plantations.

The breeding population can be thought of in terms of annually established progeny trials in which families or individual genotypes are selected for deployment in production plantations. If the genetic quality of the breeding population is defined as the mean genetic quality of progeny trials being established at a particular time, then the relationship of the genetic quality of the production population to that of the breeding population is determined by the following (see Fig. 1):

1. The proportion of the breeding population represented in the production population (for convenience, termed 'gross selection' in Fig. 1). Although maximisation of selection intensity for traits of economic importance is desirable limits are imposed by:
   - the minimum number of genotypes (families or clones) which risk considerations dictate should be represented in the production population, and
   - the size of the breeding population (i.e. the population in which selection is being made). The number of families or individual genotypes represented in the breeding population is governed by economic factors.
2. The effects of unavoidable selection for reproductive traits. It is desirable that selection as limited by the above is applied solely for economic traits. In many cases, however, selection must be applied also for traits involved in the propagation procedure. This reduces the effective size of the breeding population, and results in a decrease in genetic gain for economic traits, an effect discussed by Haines and Woolaston (1991).
3. The selection interval— the time required to identify the families or individual genotypes to be represented in the production population.
4. The multiplication interval — the time required to multiply the selected families or individual genotypes to the quantity required for operational planting. The combined effect, on the genetic quality of planting stock, of the time required for selection and multiplication was quantified and discussed by Matheson and Lindgren (1985) and the advantage of shorter intervals demonstrated.

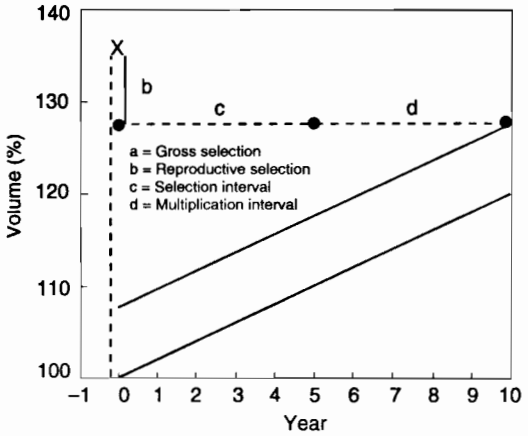

Fig. 1. Relationship of the quality of the production population (solid upper line) to that of the breeding population (solid lower line)

For time zero on the breeding population line, the corresponding position of the production population (in terms of genetic quality and time) can be calculated on the basis of the four components above, and the line representing the production population drawn parallel to the breeding population line. This involves the assumption that economic and reproductive traits are not correlated.

The slope of the breeding population line in Fig. 1 corresponds to the expected rate of gain made through breeding. The estimate of 2% per year improvement is an approximation for a hypothetical breeding program based on: the production and testing of 50 families per year; the availability of substantial variation in the breeding population; a mean generation interval of 10 years (selection at a field age of about four years, and five years required for the establishment and reliable flowering of marcotted clone banks, and a year for seed production and raising of planting stock); narrow sense heritability of 0.2, and non-additive genetic variance approximately 25% of additive. This is an adaptation from calculations made for volume gains for *Pinus caribaea* var. *hondurensis* (R. Woolaston, pers. comm.). The heritability estimate corresponds to that determined for growth traits in *A. auriculiformis*, which also demonstrates considerable variability (C. Harwood, pers. comm.).

Options for *A. mangium*, *A. auriculiformis* and the hybrid

The following propagation options have been considered for *A. mangium*:

1(a) Superior open-pollinated half-sib families, established as seedling planting stock grown from seed produced in an open-pollinated multi-clonal orchard made up of good combiners identified in the progeny trial. Some selection for flowering and seed production would be involved.

(b) Superior open-pollinated half-sib families, established as cuttings multiplied from seedling pool plants grown from open-pollinated seed collected in existing clone banks. Selection for flowering and seed production would be eased slightly by the buffering effect of the cuttings multiplication. Little or no selection for rooting would be involved.

2(a) Superior full-sib families, established as seedling planting stock grown from seed produced in an open-pollinated biclonal orchard of appropriate size. Selection for both flowering traits and an appropriate level of self-incompatibility would be necessary.

(b) Superior full-sib families, established as cuttings multiplied from seedling stool plants established with control-pollinated seed produced in existing clone banks. The use of control pollination would remove the need to select for self-incompatibility and, assuming pollen can be stored, would ease the selection for flowering phenology.

3 Individual genotypes, selected solely on the basis of phenotypic performance in the progeny trial, and propagated by multiplication by cuttings from coppice. Only a small proportion of selections would display the required multiplication rates.

4 Individual genotypes, selected as the best phenotypes within the best families in the progeny trial, and multiplied by cuttings from coppice. As above, only a small proportion of selections would display the required multiplication rates.

5 Individual genotypes, selected as the best clones in a test made up of the best phenotypes selected within the best families in the progeny trial, and propagated by cuttings from coppice. The period of clonal testing would be sufficient for simultaneous multiplication of the responsive genotypes which, as above, constitute only a small proportion of the population. It is likely that the responsive genotypes could be identified prior to establishment of the clonal test.

6 Individual genotypes, selected as the best clones in a test made up of genotypes propagated as cuttings from young seedlings randomly selected from advanced generation families (the families being established in progeny trials). As above, multiplication for mass propagation would be simultaneous with clonal testing. Assuming that the favourable responses reported elsewhere in these proceedings (Wong and Haines) continue through several further generations of cuttings, most genotypes would display satisfactory multiplication rates.

In the manner described above, and on the basis of data presented in Table 1, these options have been compared graphically in Fig. 2.

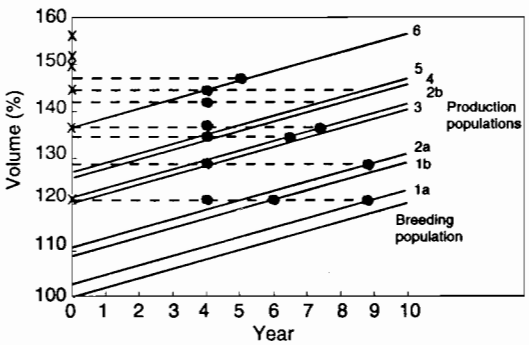

Fig. 2. Comparison of propagation strategies for *Acacia mangium*

Calculations of gains and multiplication intervals presented in Table 1 are based on the assumption that a minimum of 20 clones or 10 families would be included in a hypothetical annual planting program of 2000 ha. The annual planting stock requirement to fulfil this planting program might be approximately 200 000 plants of each family, or 100 000 plants of each clone. It has been assumed that the selection age would be four years, and that a breeding program would involve the routine establishment of clone banks of selections made. Selection intensities applied are dictated by the size of the breeding population and the calculated annual rate of replacement of families or clones in the production population (approximately one family or three clones). The balance between among, and within, family selection applied, for options involving both, is not necessarily optimal, but unlikely to be markedly suboptimal. For options involving the use of cuttings to multiply families, calculations of multiplication intervals have assumed that the production of 1000 seeds of a full-sib family by control crossing in a clone bank would be feasible, and that numbers of seeds obtainable from open-pollinated clone bank ramets would be larger. Options incorporating a clonal test involve the assumption that testing would be limited to 100 clones annually.

Table 1. Propagation options for *A. mangium*

Option	Gross advantage* (%)	Proportion which can be propagated (%)	Selection interval (years)	Multiplication interval (years)
1. (a) Superior O.P.** families from seed produced in orchard.	22	80	41	5
1. (b) Superior O.P. families, multiplied by cuttings from seed collected in clone banks.	22	85	41	2
2. (a) Superior full-sib families, from seed produced in biclonal orchards.	36	30	41	5
2. (b) Superior full-sib families, multiplied by cuttings from seed produced by control pollination in clone banks.	36	70	41	2.5
3. Superior phenotypes, multiplied by cuttings from coppice.	44	10	41	4
4. Superior phenotypes selected in best families multiplied by cuttings from coppice.	49	10	41	4
5. Superior clones selected in test comprising best families, multiplied by cuttings from coppice.	56	10	41 + 52	† †
6. Superior clones selected in test comprising genotypes propagated as cuttings from young seedlings in advanced generation families.	51	70	52	†

* Genetic superiority over mean of all families, assuming no selection for reproductive traits.
** O.P. = open pollinated
† Multiplication simultaneous with clonal test.

[1] Progeny trial

The vertical positions of the production population lines in Fig. 2 can be used to compare propagation strategies in terms of the genetic quality of production plantations being established at a particular time. This approach is similar to that applied by Matheson and Lindgren (1985) to a comparison of clonal and seed orchard options for *Pinus radiata* and *P. sylvestris*.

The cost of planting stock is a further dimension which ultimately should be considered when comparing propagation options. Apart from the assumption that control pollination would be very expensive, requiring subsequent cost dilution through a cuttings multiplication phase, no attempt has been made to incorporate costs in this analysis. It is likely though that the commercial production of cuttings would not be much more expensive than current seedling production methods.

Comparisons of propagation strategies for *A. auriculiformis* (Fig. 3) and the hybrid (Fig. 4) are based on very similar data. As a result of higher multiplication rates for cuttings of *A. auriculiformis*, selection for multiplication rate is less intense (90% of juvenile genotypes can be multiplied satisfactorily) and multiplication intervals are shorter for those options where multiplication rate is a limiting factor. The multiplication intervals for seed orchard options for *A. auriculiformis* are also shorter, by virtue of earlier flowering of marcotted ramets. The use of open-pollinated multiclonal orchards is not an option for production of the hybrid. For this taxon, it has

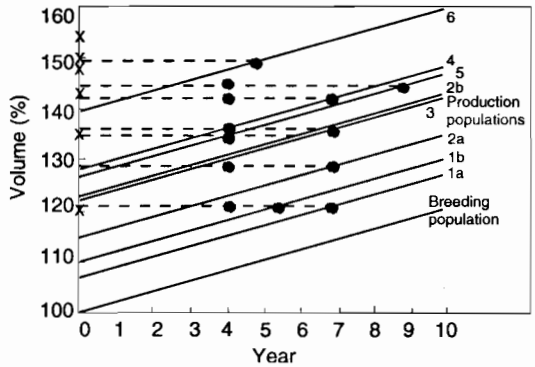

Fig. 3. Comparison of propagation strategies for *Acacia auriculiformis*

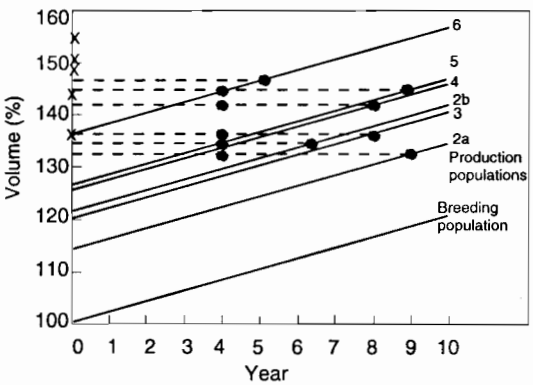

Fig. 4. Comparison of propagation strategies for the *Acacia mangium* × *A. auriculiformis* hybrid

been assumed that the biclonal orchard option would involve the exclusion of only 50% of families (since no selection for self-incompatibility would be required, assuming that selfs could be identified and culled in the nursery).

Conclusions

The use of multiplication by cuttings offers substantial advantages for the mass propagation of superior families. This advantage arises through both a shorter multiplication interval and reduction of the selection pressure for reproductive response. The analysis emphasises also the value of an effective method of control pollination to produce full-sib families. Low-budget programs, however, are likely to feature open pollination as the major approach to recombination. The analysis demonstrates that the use of multiplication by cuttings offers an advantage even for open-pollinated families.

Clonal forestry offers greater gains, depending on the approach adopted. With an active breeding program in place, the propagation of individuals selected purely on phenotype (option 3 on Figs 2–4) is not an approach which can be recommended — a family option (2b) offers greater gains. Realisation of the potential gains through clonal forestry will be dependent on more effective selection — either through the inclusion of family information in a selection index (as in option 4), or preferably through the use of data from a clonal test (as in options 5 and 6). An attractive option for these *Acacia* species is the mass propagation of clones selected in a test comprising cuttings from young seedlings of advanced generation families (option 6). In this case, multiplication for plantation establishment would be conducted simultaneously with clonal testing. For these taxa then, maintenance of juvenility as applied in this approach, is more promising than rejuvenation — selection for favourable response is less intense, and transfer of gains from the breeding to the production population is more rapid.

No immediate requirement for micropropagation in an operational propagation system is perceived. The higher multiplication rates achieved through micropropagation are not required for the propagation of either superior families or tested clones — propagation by cuttings offers adequate multiplication rates in these cases. Within reasonable limits, this conclusion holds also for larger planting programs. For the options involving clonal testing (options 5 and 6), multiplication for an annual program of 20 000 ha could still be achieved within the period of a clonal test. For family options, lengthening the multiplication interval by only a few months would be sufficient for the additional ×10 multiplication by cuttings. Micropropagation is likely also to be too expensive for direct use in the production of stock for field plantings, although this effect could be diluted by using micropropagules as stool plants from which cuttings, the final planting stock, were harvested. It is possible though that in vitro methods, compared to sequential propagation by cuttings, may offer some efficiencies for simultaneous maintenance of juvenility and multiplication.

The above applies for a trait of moderately low heritability (typical of major traits of economic importance in forestry), where clonal testing is required to identify the best genotypes. Rapid multiplication through micropropagation may be advantageous in situations where a higher heritability can be exploited:

- in those uncommon instances where a major trait for which improvement is sought demonstrates high heritability;
- where an economic trait is strongly correlated with a trait of high heritability, such that indirect selection on the basis of the latter can be used. This is also uncommon in forest tree species; and
- where selection can be based on direct recognition of DNA sequences conferring superior field performance. Advances in biotechnology may ultimately permit this.

While optimisation of the genetic quality of plantations being established is an important objective, discounted net economic returns are the primary criterion by which most forest plantation programs are evaluated. Choice of a propagation strategy must be based on both the genetic quality of planting stock and costs of the selection and multiplication procedures.

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