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Tropical Tree Seed Research

Proceedings of an international workshop held at the Forestry Training Centre, Gympie, Qld, Australia, 21–24 August 1989

***Editor*: J.W. Turnbull**

Host:
International Union of Forest Research Organisations (IUFRO) Seed Problems Working Group

Cosponsors:
Australian International Development Assistance Bureau
Australian Centre for International Agricultural Research
CSIRO Division of Forestry and Forest Products
Queensland Department of Forestry

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Foreword

As populations increase and there is greater pressure on the environment, trees and shrubs are being planted outside traditional forest areas. They play a prominent part in maintaining the sustainability of many tropical land use systems while providing timber, fuelwood, forage, tannin, medicines, honey and many other products.

The demand for high quality seed of the so-called multipurpose trees and shrubs cannot be met at present and the detrimental consequences of using poor seed can be major and long-term. The supply of tree seed of an adequate genetic and physiological quality poses a major challenge for national and international agencies and research to support this effort is particularly important. It is especially crucial to develop well based techniques to guarantee seed supplies of the many lesser-known trees and shrubs that have the potential to make a contribution to tropical reforestation programs.

Australia, in collaboration with institutions in many tropical countries, has been making a significant contribution to identifying trees and shrubs suitable for meeting the diverse needs of rural communities. The supply of seed for much of this research has been provided by the Australian Tree Seed Centre in Canberra, part of the CSIRO Division of Forestry and Forest Products. It was therefore appropriate that staff of the Division should take the lead in organising an international workshop to bring together scientists to focus on the seed problems of multipurpose trees. The workshop is the latest in a very successful series run under the auspices of the International Union of Forest Research Organisations (IUFRO) Working Group on Seed Problems. The Australian International Development Assistance Bureau (AIDAB) provided substantial support for the meeting as part of its 'Seeds of Australian Trees' project and the Australian Centre for International Agricultural Research (ACIAR) sponsored these proceedings.

The meeting provided a valuable forum for the 50 scientists who came from Australia and 22 countries throughout Africa, Asia, the Americas and Europe to share experiences and identify areas of high priority in tropical tree seed research.

G.H.L. Rothschild
Director
ACIAR

Acknowledgments

THE IUFRO Working Group on Seed Problems wishes to thank the workshop organising committee for ensuring that all facets of the four-day meeting and the post-conference tour proceeded smoothly. The workshop local organisers — Stephen Midgley, Tim Vercoe, Brian Gunn, John Doran, Kron Aken, Debbie Reynolds, Michelle Park, Ursula Schweiger, Anne Griffin, Russell Haines, Graham Applegate, Bob Pearce, Mark Dieters, Alf Said, Keith Gould, Linda Hurford, Denis Sinkovich and Greg Tilse — deserve special thanks.

The Group also extends its appreciation to Janet Lawrence for the editorial direction and preparation of these proceedings.

Opening Address

THE Queensland Department of Forestry is pleased to be involved in the organisation of this symposium on tropical tree seed, and I am personally honoured to be invited to officiate. I believe that Queensland is a very appropriate venue for a symposium on this topic, and I base this belief partly on our broad involvement in tropical forestry over many decades.

By virtue of climate, our interests have been predominantly in the use and establishment of subtropical and tropical species. Our operations have encompassed both the management of natural forests — including rainforests, wet and dry sclerophyll forests dominated by eucalypts, and the *Callitris* forests of the inland — and the establishment and management of plantations. The major taxa involved in our plantation programs have been *Araucaria cunninghamii*, and tropical and subtropical *Pinus* species and hybrids, in particular *P. caribaea* var. *hondurensis* and *P. elliottii*.

The development in Queensland of a high level of expertise in tropical forest management has been promoted by the obligatory independence from the other state forestry services, whose interests have necessarily been centred on cooler climate species, in particular *P. radiata*.

Furthermore, Australian trees, including Queensland species, are playing a major role in tropical forestry. Some have been very successful as timber species — spectacularly so in areas such as Aracruz in Brazil. However, increasingly it is being recognised that there are many Australian species of great value, or potential value, for other purposes; e.g. fodder, shade, fuelwood, oils, nitrogen fixation, and erosion control.

Not surprisingly, many of the Australian species being used in tropical areas are indigenous to Queensland. Several examples spring readily to mind:

- *Acacia mangium* and *A. auriculiformis* (and the interspecific hybrid), which have displayed considerable potential in Southeast Asia;
- *Eucalyptus grandis* — the Atherton provenances rank highly in many areas;
- *E. camaldulensis* — the Petford provenance of northern Queensland has been an outstanding performer;
- *E. cloeziana*, which is a common species in the Gympie region;
- *E. citriodora*;
- *Grevillea robusta*, a valuable timber species of the rainforests of southern Queensland, and commonly used as a shade tree over crops such as tea and coffee in tropical countries.

Historically, the Queensland Department of Forestry has made an important contribution to the use of Queensland species:

- The plantation estate includes over 40,000 ha of *Araucaria cunninghamii*. This very valuable indigenous conifer has a number of very unusual biological features. Queensland foresters take pride in the research efforts which have resulted in the development of the highly successful breeding and silvicultural approaches which underlie this very profitable plantation program.
- From the early days of this century, the identification and reservation of forested lands as state forests has been an important activity of the Queensland Department of Forestry. That the provenance representation of many species is still good owes much to the foresight of early Queensland foresters

in saving many forested areas from the very real threat of clearing for agriculture.

Many major current world problems — the greenhouse effect, shortages of fuelwood, and deforestation of the Amazon basin — are issues for which foresters must play a major role in providing solutions. As part of the challenge, it is imperative that potentially useful species be identified, distributions mapped, seed collected, and species tested in target environments. The Queensland Department of Forestry recognises its role in these activities and is responding in the following ways:

- Through cooperation with other Federal and State Government bodies, including CSIRO, we are still prominently involved in the documentation and preservation of Queensland's flora.
- We have a seeds section involved in the collection, storage and distribution of seed, and also cooperate with the Australian Tree Seed Centre in their collections. Having been heavily involved in provenance testing of a range of both exotic and indigenous species, we fully appreciate the importance of systematic collections and proper authentication of seed collected.
- Plantings established, maintained and assessed by the Queensland Department of Forestry represent a key component of ACIAR's important international trials of Australian species.

Australia has been very generous in the sharing of genetic resources with respect to indigenous forest species. However, it is important also that *expertise* gained here in the silviculture and use of tropical Australian species be made available elsewhere.

By virtue of its long and unique involvement in tropical forest research, silviculture and management, the Queensland Department of Forestry has been actively involved in forest research and development in other tropical and subtropical areas, including southern China, Vietnam, Malaysia, Vanuatu and Fiji. These projects have included the testing of Queensland species. It is worth mentioning that the widespread interest in *Acacia mangium* stems from its introduction to Malaysia, for which an officer of the Queensland Department of Forestry, Mr Don Nicholson, was responsible.

Many advances are being made in approaches to the systematic collection, distribution and testing of Australian species. It is appreciated that much remains to be done, and that the Australian Tree Seed Centre and also the Queensland Department of Forestry must continue to play an active role in the collection, preservation and documentation of tropical species.

Ultimately, however, until other efficient methods for the storage and multiplication of germplasm are available, we are heavily dependent on the availability of reliable methods for the collection, storage and germination of seed. For many of these biologically quite diverse species, this is a major obstacle to testing and exploitation. There are some major problems to be overcome, for example the storage of seeds of the 'recalcitrant' species, and the definition of optimal methods for the germination of seed of many species.

I believe that this symposium will provide an excellent forum for discussions on these problems, and I take great pleasure in officially declaring the symposium open.

Norm Clough
Assistant Conservator
Division of Technical Services
Queensland Department of Forestry

Introductory Paper

Tree Seed in Forest Development

S.J. Midgley*

ON BEHALF OF the organising committee I would like to welcome you to this IUFRO meeting on seed problems of tropical and subtropical multipurpose tree species.

The supply of high quality seed is central to the success of tree planting projects. It costs just as much to establish trees from poor seed as it does from seed of the highest genetic potential but the difference in material and economic returns can be great. The question of the assured supply of quality seed and the problems that this can cause forestry development projects have been well addressed several times in recent years. Turnbull (1984), Burley and von Carlowitz (1984), Burley (1985), von Carlowitz (1986), Ayling (1987) and Turnbull (1987) all stressed the intrinsic importance of quality tree seed.

The problems are particularly apparent with lesser known species, where adequate certified seed is simply not available, and for species with wide natural distribution where availability of a wide genetic range presents logistic problems. Adequate information on requirements for the maintenance of physiological and genetic quality of seed is basic to the silvicultural development of these species. The importance of seed-related research is clear.

Increasingly, national governments have recognised tree seed supply as a development issue and donor agencies have been encouraged to assist the development of tree seed centres at both the national and regional level. Regional centres in Harare (for southern Africa) and Thailand (ASEAN) are now functioning as are national centres in other countries including Kenya, Nepal, Burkina Faso and Ethiopia.

However, despite this increased activity and despite the recognition that failure to secure the best possible seed may influence the success or failure of a tree planting project, seed matters are often considered peripheral to mainstream forestry activities. It is an unfortunate fact that supply of quality seed is often overlooked in project planning. Seed purchase is often the responsibility of clerical staff who generally use simple criteria for seed purchase, e.g. the lowest quote. Orders for bulk lots of seed are still received in Australia with no reference to provenance or requirement for origin details — despite the vast resource of data demonstrating that growth rates and other characters can vary greatly between provenances, for example *Eucalyptus camaldulensis* (Midgley et al. 1987). Seed cost is a small proportion of establishment cost (Table 1) and it is clear that projects can afford to purchase the best seed rather than the cheapest.

Against this background we can see how minor the extra cost for quality seed can be, especially when it can greatly influence the project outcome. Added weight is given to this aspect if the seed used is to provide the base for subsequent tree improvement and breeding programs.

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Table 1. Tree seed costs in plantation establishment.

Species	Approx. cost/kg (A$)	Average no. seeds/kg	Approx. no. of plantable seedlings (1:4)	(*1:3)	Ha/kg (at 3 m × 3 m)	Approx. cost/ha (A$)	Proportion (%) of total establishment costs (estimated A$1000/ha)
Eucalyptus camaldulensis	200	670000	167500		168	1.19	0.1
E. grandis	200	652000	163000		163	1.23	0.1
E. globulus ssp. *globulus*	400	73500	18375		18	21.77	2.2
E. dunnii	600	237000	59250		59	10.13	1.0
E. nitens	800	264000	66000		66	12.12	1.2
Acacia mangium	400	70000		23333*	23	17.40	1.7
A. auriculiformis	600	50000		16667*	17	36.00	3.6

(A$ 1.00 = US$ 0.75)

Collection and supply of seed from native forests is seasonal and variable so projects should allow adequate lead time for seed orders. Seed is sometimes mistakenly viewed as a free commodity. Those of us responsible for the management of seed centres realise just how expensive it is to finance the collection, storage, testing and dispatch of seed. Projects pay for petrol, labour, equipment and supplies — why not seed?

I would like to share some anecdotes regarding the consequences of lack of regard for supply of tree seed.

- A large quantity of *Acacia auriculiformis* was required for a project in southern China. Quotes were called for in Australia in the expectation that seed of Australian origin could be provided, however, no provenance or origin details were specified. The Chinese body accepted the cheapest quote from an Australian supplier and eventually took delivery of seed which had originated in India. The growth and form of the trees was far below expectation. The lessons we can learn from this are: 1) specify the details you expect to accompany seed; 2) do not accept seed of unknown origin.
- In Sri Lanka some 10 years ago the cheapest seed of *E. camaldulensis* was supplied to a project. Unfortunately the provenance supplied was from the southern part of its range in temperate Australia and unsuitable for the seasonally dry tropical conditions of northern Sri Lanka.
- In the semi-arid areas of Kenya, *Acacia aneura* was included in trials. A seed dealer in Europe supplied the seed with no origin details. This species is one of the most widespread throughout Australia's arid zone and demonstrates considerable variation in growth form, fodder value and morphology. Researchers wishing to repeat the experiment on seeking seed for wider plantings will experience great difficulty. Again the lesson is never to accept seed without origin details.

The message that we as a group of seed scientists, can take from this meeting to our national governments and stress to donor agencies is that supply of high quality seed is central to any tree planting program. Seed-related forestry work deserves the investment of increased skills and resources if it is to fully serve the international priorities of reforestation.

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Seed Biology

Seed and Pollen Cone Production in *Larix occidentalis*

R.C. Shearer*

Abstract

A 5-year study in Idaho, Montana, Oregon and Washington USA was started in 1985 to determine why few western larch (*Larix occidentalis* Nutt.) seed cones mature in most years. The frequency and amount of seed and pollen cone production were quantified and major factors of seed cone mortality were identified. Seed cones were initiated on most study trees each year, but few matured. Frost killed most seed cones in 3 of the 4 years. Insects caused high cone mortality or seed loss within mature cones when frost was not a major factor. Pollen cones occurred on all study trees each year, but their survival was not determined.

WESTERN larch (*Larix occidentalis* Nutt.) is a favoured multipurpose species in the forests of inland northwest USA. Its luxurious light green foliage of spring and summer that turns golden in the autumn pleases the eye, its rapid growth produces wood prized for lumber and plywood, and its firewood is in demand. Based on cone crop reports submitted by district forest rangers and observations by research personnel, larch was rated as a good cone producer in western Montana, from 1908 through 1953 (Boe 1954). Reports are not available for other areas, but foresters claim that larch produced few mature cones for many years through much of Idaho, Oregon, and Washington. Reasons for poor cone crops were uncertain before 1985, although insects (Shearer 1984) and frost (Shearer 1985) were known to reduce cone and seed production.

A 5-year study began in 1985 to quantify larch seed cone potential and to identify major factors decreasing its cone and seed production. Cone and seed production on larch originating after wildfires earlier this century have been monitored from 1 to 4 years at 14 sites. Nine study areas are located in Idaho, two in Montana, two in Oregon, and one in Washington. This paper summarises seed and pollen cone data only from the nine stands (seven in Idaho and two in Montana) with 4 years of data (Table 1).

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Methods

At each location, 10 open-growing dominant or codominant larch trees were chosen for study (except for five trees at Savage Camp — the youngest stand) using these selection criteria: (1) crown length at least 40% of the total tree height; (2) accessibility for climbing; (3) evidence of prior cone production. Each sample tree was climbed using a combination of tree-climbing ladders (first 6 m), steps attached to the open bole (between the top ladder and the lowest live whorl of branches), and free climbing on live branches (to about 9 cm bole diameter). The living crown was divided into thirds, and the number of branches originating at the bole within each third was recorded.

Each year, in the spring, the study areas were first visited when roads were free of snow. At this entry, the number of new seed cones on the 10 sample trees was quickly estimated using binoculars. Then the five trees with the greatest cone counts were climbed and the number of seed cones was more accurately estimated by counting (1) the total number of branches for each third of the live crown, (2) the number of cone-bearing branches by crown thirds, and (3) the number of new cones (living and dead) on six randomly-

Table 1. Location and elevation of nine study areas, and average age, height, and diameter of 10 western larch sample trees (five trees at Savage Camp).

Study area	Location		Elevation	Average		
	North lat.	West long.	(*m*)	Age at 15 cm (*yr*)	Total height (*m*)	Diameter at 1.4 m (*cm*)
Meadow Cr.	48°46′	116°14′	762	80	27.3	33.8
Standard Cr.	48°04′	115°31′	1,097	68	21.6	25.1
Twin Cr.	48°04′	116°09′	853	82	30.2	39.1
Twelve Mile Cr.	47°28′	115°17′	1,372	65	23.2	28.2
Beacon Light	47°27′	115°40′	1,189	83	30.6	38.4
Savage Camp	46°29′	115°32′	1,463	46	23.9	32.5
Ericson Ridge	45°54′	115°29′	1,433	55	23.2	34.0
Peter Ready Cr.	45°39′	116°03′	1,768	64	23.4	33.0
Brush Mountain	45°09′	116°22′	1,524	63	24.9	34.0

selected branches — two from each third of the crown. The number of potential cones on each tree was estimated by multiplying the average number of cones per sample branch by the total number of cone-bearing branches. Seed cone survival was estimated in August by counting the number of cones that matured on the six randomly selected branches. Cone mortality was determined by subtracting surviving cones from the total cones obtained at the first visit of the year.

Cone development and the time and cause of cone damage were documented for the two larch with the most cones by recurring observations made on 25 cones marked during the first examination. Cone length and damage were noted at each visit. Dead cones were removed and probable cause of death identified.

When available, 50 mature cones were picked at each study area in late August for analysis. From each sample lot, 10 cones were randomly selected for detailed study, then placed in separate paper bags and allowed to dry and open. Prior to analysis these cones were heated to 43°C for 24 hours. Cone analysis consisted of dissecting the cones and counting the number of lower infertile scales, upper infertile scales, and fertile scales. After accounting for the seeds from each cone that were damaged by insects, the remainder were x-rayed and categorised as abnormal, damaged, empty, malformed, or filled. Then all but the damaged seeds were placed in germination boxes, moistened with water, stratified at 0°C for 30 days, and incubated 3 weeks for germination.

Results

The average number of live branches originating at the bole on the five sample trees at each location increased by 11 new branches from 1985 to 1988 (Table 2). In 1988, 46, 34 and 20% of the branches were located in the upper, middle, and lower thirds of the living crowns. Seed cone buds opened on 55% of the branches in 1985, 50% in 1986, 56% in 1987, and 61% in 1988. But only 11, 1, 45, and 13% of the total branches had matured cones those years. The lowest elevation site not only had the greatest number of branches with surviving cones each year, but had more than twice the number of branches with mature cones than the other eight sites combined. Pollen cones developed on 85, 77, 80 and 83% of the branches in 1985, 1986, 1987, and 1988.

All larch study trees produced seed cones each year except at Savage Camp. There the trees averaged 9–37 years younger than at any other site (Table 1). Only one of the five trees produced cones each of the 4 years. The other four trees produced cones one to three times during the 4-year period.

An average of 83% of the seed cones was produced in the upper two-thirds of the living crown and only 17% within the lower third of the crown. The number of potential and mature cones per tree varied greatly between sites (Table 2). The number of potential cones ranged from 163 to 1270 in 1985, 24 to 2839 in 1986, 2 to 2108 in 1987, and 44 to 3029 in 1988. The number of mature cones ranged from 0 to 173 in 1985, 0 to 41 in 1986, 0 to 818 in 1987, and 0 to 1061 in 1988. Only 6% of the potential cones matured in 1985 (0–16%), 0.5% in 1986 (0–73%), 52% in 1987 (<1–95%), and 10% in 1988 (0–78%).

During the first four seasons, more than six times the number of cones matured on open-growing trees within the plots than on comparable trees growing within the adjacent closed stands (Table 2). In 1987, an average of 68 cones matured

Table 2. Summary of western larch cone and seed data for nine study areas for 1985, 1986, 1987, and 1988 — average and standard error of the mean.

Data collected	1985		1986		1987		1988	
	Ave.	s.e.	Ave.	s.e.	Ave.	s.e.	Ave.	s.e.
	Average number per plot tree							
Total live branches	130	3	135	3	138	3	141	3
Branches with at least one pollen cone	110	3	104	5	111	5	117	4
Branches with at least one seed cone								
Potential, spring	71	6	68	6	77	6	86	5
Mature, August	14	4	2	1	62	5	18	4
Pollen cone ('000)	7.2	.6	4.2	.5	5.8	.6	5.6	.6
Number of seed cones								
Potential, spring	656	101	877	148	625	91	955	146
Mature, August	38	12	4	2	327	48	115	44
	Average number of seed cones per tree by crown condition							
Open crowns	17.0	4.1	1.0	.4	68.2	9.9	55.7	14.6
Closed crowns	1.1	.8	.4	.4	17.0	6.8	4.2	2.9
	Average number of seed per cone							
Total	104	2	117	2	118	1	117	2
Empty	48	2	53	5	69	1	48	4
Insect damaged	43	4	44	5	7	1	43	4
Aborted	9	1	9	1	3	<1	18	2
Malformed	2	<1	<1	<1	3	<1	1	<1
Filled	10	1	11	1	36	1	7	1
Germinated	9	1	10	1	35	1	7	1
	Per cent of filled seed							
Germinated	94		99		94		93	

on 443 larch trees growing on plots within the open-crowned stands, compared to an average of only 17 per tree within the uncut forest stand.

Pollen cone production was greatest in 1985 (Table 2), ranging from 900 to > 16,000 per tree. In 1986, pollen cone production ranged from 80 to 13,100; in 1987 from 0 to > 28,600; and in 1988 from 152 to > 17,300. About 45% of the pollen cones were produced in the upper third of the live crowns, 40% in the mid third, and 15% in the lower third of the trees during the first 4 years.

Most seed cone buds opened by late April. Cone elongation was rapid, reaching two-thirds of the mature length by early May and nine-tenths by late May. In 1987, mature cones averaged 27 mm (range 18–36) in length and had 59 (23–80) fertile and 8 (7–11) infertile scales.

Seed cone mortality was high each year except 1987 when the study sites had low cone potential. Most mortality occurred in April and May during the period of rapid cone growth. Frost caused the greatest loss of seed cones during this study (Shearer and Theroux 1986). Only the two lowest elevation sites usually escaped frost damage.

Insects caused most of the other seed cone losses each year. The larch cone maggot *(Strobilomyia laricis)* was identified as the major cone-feeding insect on all the study plots (Jenkins and Shearer in press). Other insects causing damage were the western spruce budworm *(Choristoneura occidentalis)*, a woolly aphid *(Adelges viridis)*, and scale midges *(Resseliella* sp.).

A high proportion of seeds within the mature cones were not viable (Table 2). These seeds composed from 54% (1987) to 94% (1988) of the total seed during the first 4 years. Empty seeds made up 38% (1985) to 58% (1987) of the total seed. Insect pests often damaged a high proportion of the seeds (both filled and empty), ranging from 6% in 1987 to 41% in 1985. Abnormal (aborted and malformed) seeds made up from 5% (1987) to 16% (1988) of the total seed.

Larch seed germinates easily after stratification. Most germination occurred within 5–6 days after incubation started, and ceased after 8–10 days. Germination ranged from 93 to 99% during the first 4 years of this study (Table 2). Seeds that were not viable came mostly from individual cones rather than being distributed throughout the sample. Poorest germination (93%) occurred in 1988 — the year of the lowest average number of filled seeds, and highest germination (99%) occurred in 1986 — the year of lowest cone survival.

Discussion

There is a critical need for larch seed for growing planting stock and for direct seeding.

Years of low cone production have also limited replenishing seed reserves. Potential seed cones are produced every year on most good-vigour, open-growing larch trees, but high cone and seed mortality severely limit the amount of viable seed that is available. Frost (3 of 4 years) and insects (1 of 4 years) were the major causes of low cone survival in this study. Because larch buds open 2–3 weeks ahead of other conifers, frost may eliminate the larch cones but have little or no influence on the potential cone crop of other species. Often a single occurrence of the air temperature falling below -4°C soon after bud burst killed all larch cones. In areas highly susceptible to frost-kill, resource managers should plan to collect seed cones each year they mature to help replenish seed reserves. When a cone crop is identified in the spring, another examination should be made to assure sufficient cones are available to justify collection.

Poor cone production also limits the potential for natural regeneration of larch, despite appropriate site preparation and favourable conditions for its establishment. Without some planting, larch may be poorly represented in new stands even if the reserved seed source has a high proportion of larch.

This study shows the wisdom of locating larch seed orchards and seed production areas in the most frost-free areas for greater cone survival. Established seed production areas should be re-evaluated to determine if their location provides a reasonable chance to obtain cones.

These results suggest alternatives to meet the needs for larch seed — similar techniques may also enhance seed production on multipurpose tropical or subtropical species. (1) Produce genetically superior seed indoors, safe from damaging factors. (2) Manage orchards and seed production areas in favourable environments. (3) Girdling and implanting with insecticides to stimulate and protect seed production. (4) Rooting of stem cuttings from seedling ortets of seed lots in short supply. (5) Bagging of strobili to reduce effects of damaging factors. (6) Topping highly fecund trees to concentrate seed production nearer the ground and simplify collection.

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Sugar Palm (*Arenga pinnata*) and its Seed Problems in Indonesia

Masano*

Abstract

The sugar palm, *Arenga pinnata*, has potential for small farmers as a multipurpose tree species. Tapping the sugar palm is a source of extra income. Some seed problems related to the cultivation of sugar palm are seed collection, germination pretreatment and seed storage.

SUGAR palm (*Arenga pinnata* Wurmb) has an important role in rural communities. Through cultivation of sugar palm they can increase their income.

Most of the sugar palm can be utilised — the young leaves for cigarette paper, old leaves for roofing, the black fibres for brooms and brushes, male flowers tapped for sugar sap, and immature fruits eaten by people.

Gunawan (1988) states that the main products of this tree are sugar, sago, saguer and alcohol. In North Sulawesi one stem produces ca 200kg of sago. The outer part of the trunk is very hard and used for barrels, flooring, furniture and tool handles. Other uses include honey production and medicinal products from the roots.

Sugar palm is commonly tapped to produce sweet sap. Sap production depends on many factors including climatic conditions, age of tree, and the length of time the sap has been running. (Brown and Elmer 1919).

The seeds of immature fruit when carefully extracted and boiled in sugar make a pleasant confectionery which is prepared commercially (Whitmore 1979).

In Indonesia *A. pinnata* is not widely cultivated. One of the reasons is lack of information on how to germinate sugar palm seeds.

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Distribution

A pinnata formerly known as *A. saccharifera* Labill (Heyne 1929; Brown and Elmer 1919), grows as a solitary specimen 12–15 m high and about 40 cm in diameter (Whitmore 1979; Brown 1919).

The native habitats of *A. pinnata* in South and Southeast Asia are tropical rain-forest to dry forest. It occurs from sea level to 1200 m elevation (Johnson 1987).

In Indonesia *A. pinnata* grows in the forest and also in village gardens. It grows on most islands of Indonesia e.g. Java, Sumatra, Kalimantan, Celebes, Moluccas, Irian Jaya and Lesser Sunda islands.

Regeneration

Natural regeneration of *A. pinnata* occurs when ripe fruits fall and are eaten by animals such as wild pigs, fruit bats and civet cats (Brown and Elmer 1919). After eating the fruits, the seeds pass through the animals unharmed. Distribution of seedlings thus depends on the activity of the animals.

Sugar palm is widely cultivated in some areas e.g. India, Southeast Asia (Johnson 1987). It is sometimes planted after shifting cultivation.

Seedlings for planting out can be obtained from the wild from natural regeneration or raised as nursery seedlings. Pretreatment of the seed to accelerate germination in the nursery is necessary.

Seed Handling

Flowering and Fruiting

Times of flowering and fruiting of *A. pinnata* are uncertain. Whitmore (1979) recorded that *A. pinnata* began flowering 6–12 years after planting. Burkill (1935) recorded that *A. pinnata* begins to flower after 7–10 years. When planted at higher altitudes (1000m) it begins to flower after 16 years, but at the lower altitude (600m) it will flower after 12–13 years (Heyne 1929).

Time of fruit maturation is also hard to determine exactly. It is estimated that in Indonesia sugar palm fruit ripens 2 or 3 years after flowering. Fruits of *A. pinnata* are round, about 5 cm in diameter and contain 2 or 3 seeds (Brown and Elmer 1919). Each tree has 4 or 5 inflorescences.

The exocarps of young fruits are green and gradually change to brownish-yellow when mature. The endocarp is brownish-yellow when young and changes to black at maturity. Old seed is hard, about 2.5–3.5 cm long and 2–2.5 cm wide.

Seed Collection

Successful establishment of plantations requires seedlings raised from high quality seed. To collect quality seed requires information on the sequence of fruit maturation and dispersal. Seeds are collected from fruit which is still on the tree.

Seed Production

Heyne (1929) stated that the sugar palm produces 4–5 inflorescences. From my observations, the average number of fruits per inflorescence is around 480. Each tree contain 4–5 inflorescences, each fruit has three seeds, thus every tree produces 5000–7000 seeds.

Some problems of seed production are: immature fruits usually collected for making sweetmeat; the tree only produces fruit once; mature fruit commonly eaten by animals. Whitmore (1979) stated that the sugar palm tree dies after flowering and fruiting at about 30 years. Gunawan (1985) indicates that trees are cut for sago when older than 30, suggesting a longer lifespan than given by Whitmore.

Seed Extraction

The outer part of the fruit contains a stinging crystal which is exceedingly irritating. Blanco (Brown and Elmer 1919) related how, in former times, the fruits were thrown into water and allowed to decay, resulting in a fluid which causes intense itching and burning wherever it comes in contact with the skin. Contact between the seed coat and the skin is harmful. Extraction of *A. pinnata* seed is thus hazardous, and hands must be protected by rubber gloves.

Table 1. Methods for extracting *Arenga* seeds from collected fruit.

Treatment	% decay fruits	Notes
— Cover with moist top soil	73	Observe after 24 days.
— Cover with moist leaf litter	73	Irritating effect will decrease after exocarp decays.
— Fruit placed in black plastic bag with 100 g carbide	14,8	
— Fruit only placed in black plastic bag	3,5	

Arenga seeds can be extracted as shown in Table 1, but further research to refine these techniques is necessary.

Seed Pretreatment

Germination of *Arenga* seeds is slow, usually 30–50 days after sowing. Pretreatment is necessary to accelerate germination.

One method of accelerating the process is to scarify the seeds by filing near the embryo. Further research is need to improve current pretreatment techniques.

Seed Storage

Viability of *Arenga* seed is short. Seeds will not store for more than 2 months. After this time the percentage of germination decreases gradually so that after 3 months the germination rate is only 25%. Research on seed storage methods is required.

Conclusions

Although *A. pinnata* grows naturally in the forests of Indonesia, it can also be grown in plantations or intercropped with food crops in agroforestry systems. The production and processing of sugar, sago, alcohol and various other products will increase farmers' incomes and rural employment. Promotion of this multipurpose tree is therefore highly desirable. However, the collection, handling and storage of *A. pinnata* seeds have been little studied and lack of suitable techniques will be a constraint to more extensive

planting of this species. Further research on seed handling techniques is therefore a priority.

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Attacks by Spermatophageous Insects on the Seed of Three Acacias — *A. albida, A. nilotica* var. *adansonii* and *A. senegal*

H. Sary and L. M. Some*

Abstract

The study of 75 fruits taken from three levels of the crown (lower, middle, upper) of 5–10 specimens of *Acacia albida*, Del. *Acacia nilotica* var. *adansonii* (Guill. et Perrott.) O. Ktze, *Acacia senegal* (L.) Willd. produced the following observations:

- for *A. albida* seeds the adults and larvae of Bruchidae parasitise the endosperm of the seeds.
- for *A. nilotica* var. *adansonii* the unripe fruits (green) have caterpillars as parasites, whereas the dry fruits (or those about to dry) are attacked by larvae and adults from several bruchid species, including *Callosobruchus maculatus*.
- for seeds of *A. senegal*, we noted holes in the fruit valves (still green), malformation of seeds as well as parasitism of seeds by larvae and adults of Bruchidae.

THIS study was prompted by the high rate of insect parasitism noted in the seeds treated by the Centre National de Semences Forestières (CNSF). The aim of this preliminary study, which involved the priority species (CNSF 1985) *Acacia albida, A. nilotica* var. *adansonii* and *A. senegal*, was to provide information on the attacks by spermatophageous insects on the seeds which are still on the mother-trees. The study lists types of insects, nature of damage, area of attack on the seed, and preferential area of development of parasites on the various levels of the tree.

Method

Observation sites were selected by species — *A. albida* at Kongoussi *A. senegal* at the Yabo classified forest (both of the two sites are situated at about 100 km north of Ouagadougou) and *A. nilotica* var. *adansonii* at Boussouma, situated about 100 km northeast of Ouagadougou.

The studies which took place in November 1987, involved five trees of *A. nilotica* and 10 trees of both *A. albida* and *A. senegal*. The average heights of the selected trees bearing seeds were 6, 15 and 5 m respectively for the three species. Selection criteria of these trees were: the fruit-bearing state (abundance of fruit, ripeness of fruit), the accessibility of fruits (tall trees eliminated) and the distance between trees.

The crown of each tree was divided into three levels (upper, middle, and lower). Scientists collected 25 fruits from each level and from each specimen tree.

The following studies were carried out on the collected fruits:

— presence of parasites;
— nature of parasites;
— the area of attack (fruit tissues or seed tissues);
— the rate of parasitism.

Results

Acacia albida

At the time the fruit was collected (late February) it had already matured on the trees. Holes were noted in the seedcoats, and larvae and adults were observed in the seeds. The evidence of

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insects (present or former) was revealed by holes observed in the pod. Counts of the number of holes and the number of seeds attacked per pod revealed that the latter is almost always higher than the former. The perforations correspond to the exit holes of adults.

The observation of the surface of pods revealed the presence of small swellings. These are generally just at the level of the seed which is already spoilt. This proves that the penetration of the insects is prior to the maturation of the pods. The fresh tissues made it possible to fill in the entry holes.

The main area of attack is the endosperm of the seeds—this part is eaten by the larvae and adults of the bruchids. The first bruchid species collected was similar to *Caryedon intersticus*, already identified by Some (1984). The genus of this bruchid is characterised by the presence of cocoons (pupae) outside the seeds. The presence of another species of bruchidae, *Bruchidius uberatus (Fahraeus)* has also been observed.

Table 1 shows the percentage of seeds attacked per tree and per level.

Table 1. Percentage of *Acacia albida* seeds showing damage due to insects.

Tree No.	Level Lower (%)	Middle (%)	Upper (%)	Average per tree (%)
1	62	77	57	65
2	62	72	79	71
3	46	48	29	41
4	79	68	73	73
5	71	65	61	66
6	25	52	57	45
7	40	32	28	33
8	44	71	71	62
9	49	61	65	58
10	22	18	24	21
Average per level (%)	50	56	54	53

Acacia nilotica var. *adansonii*

The fruits harvested showed a variable ripeness from one fruit to the other and from one tree to another. Thus, some were very dry while others were still very watery. Among the immature fruits, there was not much difference between fruits with well-differentiated and well-formed seeds and fruits with developing seeds. The nature of attack varied according to the degree of ripeness.

Unripe Fruits These are fruits with seeds which are still green and watery. The presence of insects is manifested externally by the black puncture points on the pod. We observed larvae which were completely different from the Bruchidae larvae. These were *Chilades cleusis Denais*, green lepidopterous caterpillars that live in the watery tissues of the fruit or of the seed and arrest seed development.

Mature Fruits In this case, the fruits are dry and therefore the seeds are dry with a prominent endosperm (whitish in colour). There are also holes on the fruit pods. The insect *Callosobruchus maculatus* (Some 1984) lives as a parasite in the seed and eats the endosperm.

There is an intermediate stage — a period when fruits are in the process of drying, seeds are already formed and the endosperm is well differentiated. Caterpillars were found in such fruits.

No chrysalid was noted in the fruits. It is difficult to say from this whether the nymph stage occurred in the seed or not.

Some (1984) had already identified *Callosobruchus maculatus* on the *Acacia nilotica* and Ouedraogo (1978) recorded *Callosobruchus maculatus* parasitism on leguminous seeds. Another species of Bruchidae was also observed but not identified. Let us note that Varaigne-Labeyrie and Labeyrie (1981) also described *Caryedon intersticus* on *Acacia nilotica* seeds.

The percentage of seeds attacked is given in Table 2. This includes the seeds spoilt by the caterpillar as well as those attacked by Bruchidae.

Table 2. Percentage of *Acacia nilotica* var. *adansonii* seeds showing damage due to insects.

Tree No.	Level Lower (%)	Middle (%)	Upper (%)	Average per tree (%)
1	52	30	51	44
2	43	44	48	45
3	33	62	37	44
4	21	33	35	30
5	73	63	70	69
Average per level (%)	44	46	48	46

Acacia senegal

The dry valves, which had often separated, were collected. There were no insects, only the evidence of their passage in the fruit (Table 3). Three types of damage can be described:

- Fruits showing valves with holes, the contours of the holes, which are symmetrical i.e. identical on the two valves, are jagged (with

Table 3. Percentage of *A. senegal* seeds showing damage due to insects.

Tree No.	Level Lower (%)	Middle (%)	Upper (%)	Average per tree (%)
1	12	13	20	15
2	7	20	18	15
3	73	86	97	85
4	25	22	19	22
5	11	14	26	17
6	17	13	26	19
7	8	27	6	14
8	28	19	18	22
9	22	24	25	24
10	20	30	24	25
Average per level %	22	27	28	26

excrescences), proof of the action of an insect which had eaten the valves, probably when they were still green, tender and well-joined together. Sometimes, the tissues had been eaten on the valve borders.

- Seeds showing malformation, abnormally big seeds with one or several excrescences. For some seeds, there were up to four excrescences:
- Seeds with holes and emptied by the spermatophageous insects.

No seed contained any insect (larva or adult) at the time of study. The relatively small dimensions of dry seeds compared to the size of most Bruchidae (which were certainly responsible for this attack) does not easily permit a permanent presence of the insect in the seed. The question therefore is whether the attack is only the work of adult insects.

Moreover we observed many incompletely developed seeds, which led to the conclusion that the ovules had aborted. These seeds were generally on either extremity of the fruit.

Prospects

It has been clearly shown that seeds of the three species studied can be heavily attacked while on the mother-tree before they are collected, during the ripening period of the seeds. This, naturally, affects seed quality as well as the quantity of seeds collected during one season. The study of insect attacks on the seeds of these species and other leguminous trees, needs to be intensified. This study could be as follows:

- Cataloguing of insects harmful to seeds on the seed tree and identification of various insect species according to ecological zone and by plant species.
- Study of the course of development of the parasites according to the phytogeographical zones and the state of maturity of the fruit. It is therefore important to know the period of oviposition on the fruit.
- To determine the preferential area of development of parasites according to the various levels of the tree, and it would be useful to compare the rate of parasitism from the outside towards the interior of the crown.

These data will be fundamental in the improvement of the health of the seeds on the mother-tree.

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Seed Development and Germination Responses of *Melia azedarach* var. *australasica*

M.W. Moncur and B.V. Gunn*

Abstract

Phenological patterns of *Melia azedarach* growing in Canberra and Atherton, Australia are presented. The complete cycle from bud burst to ripe fruit drop took ten months at Atherton and twelve months at Canberra. Viable seeds were obtained from green fruit which had reached maximum dry weight. Later the fruit turns orange/yellow at leaf drop and this colour change can be used as a visual indicator of maturity.

High temperatures, above 30°C, are required for good germination. No germination was recorded below 24°C. Removal of flesh improved germination and emergence. Storage of fruit or endocarps at low temperatures (3°C) had no effect on germination even under high moisture conditions.

MELIA azedarach is a native of Asia and selected forms have been long cultivated in both India and China. Groups of cultivars have been selected from these two centres of domestication and introduced to other warm parts of the world (Mabberley 1984, Fig. 1). It is one of the most widely cultivated of all tropical trees, sometimes becoming naturalised, notably in West Africa. It has been difficult to separate naturalised cultivars and wild specimens and, as Mabberley (1984) has pointed out, with the widespread cultivation of the tree and destruction of the original forest, it may now be impossible to reconstruct this history anyway.

The earliest reference to the species is on a stela of the Assyrian king Assurnasirpal II, dated ca. 879 B.C. It was first mentioned in Chinese literature in 300 B.C. Stocks in Europe and North America seem to have two distinct origins: India via the Middle East (and via Sri Lanka and the Netherlands) as early as the 16th century; and China via Japan in the 19th century. It is widely naturalised in the southern United States (*M. floribunda*) with a mutant known as the Texas Umbrella Tree. Fast-growing forms are planted for forestry in South America including var. *gigantea*. In Africa the naturalised form is *M. guineenis* while *M. volkensii* and *M. bambolo* are considered indigenous. In the Pacific it has been difficult to separate *M. dubia* and var. *australasica* (White Cedar) from *M. azedarach* (Persian Lilac).

After a taxonomic revision, Mabberley (1984) has treated the whole complex as one species (*M. azedarach*) comprising the wild populations and the two major groups of horticultural cultivars. He suggests that those cultivated plants which are considered distinct should be given cultivar names by those who deal in the horticultural trade.

The species has many uses: its timber for veneers, cabinet work and joinery; as a source of domestic firewood; its seeds are made into beads and rosaries and it is widely grown as an ornamental (Turnbull 1986). Vercoe (1987) has demonstrated its leaf fodder potential and it is fairly tolerant to salinity (Zwar 1975). It has some limitations in that its fruit may be toxic to man and pigs (Everist 1969) and it has potential to become a weed.

Melia azedarach is one of the few Australian native trees that is winter-deciduous. It occurs naturally from northern Queensland to southern

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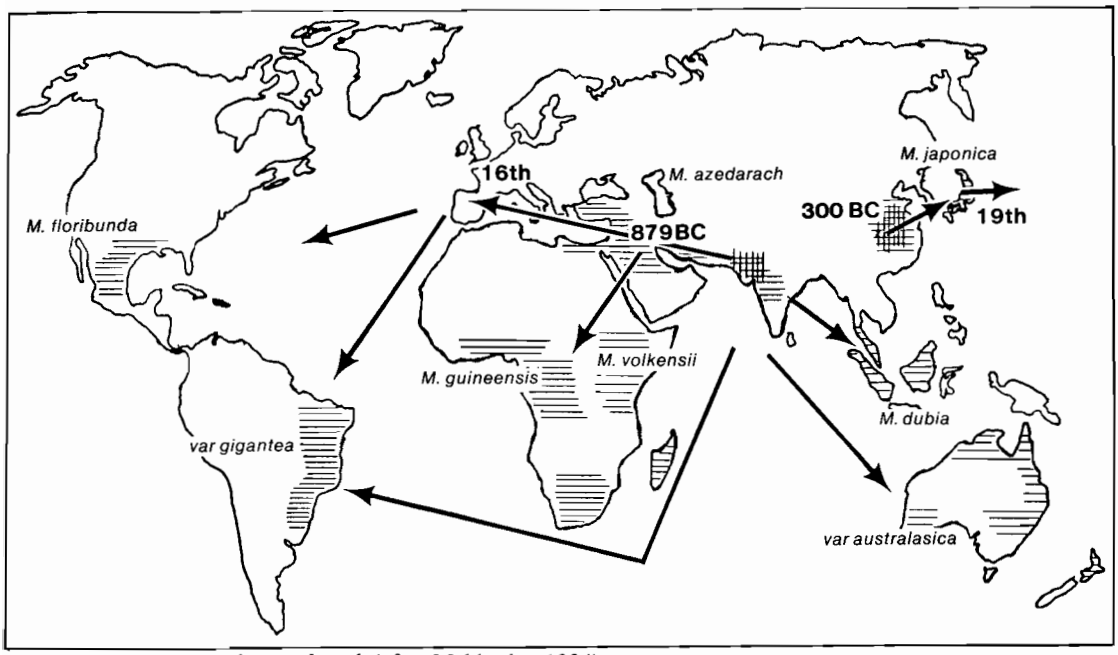

Fig. 1. Dispersal of *Melia azedarach* (after Mabberley 1984).

New South Wales, usually within 100 km of the coastline (Turnbull 1986). Plant specimens in the CSIRO Herbarium collected by Brown at Mount Hunter near Port Jackson in 1802–5 suggest its naturalisation in Australia preceded European settlement. Seed has since been dispersed, through its use as an ornamental, to many inland towns and districts often much hotter and drier than its natural location (Fig. 2).

It is important for seed collectors to know when the fruit is mature, whether viable seed can be harvested while still attached to the tree and how long and under what conditions the seed can be stored. Low seed germination has been reported, with less than 5% from freshly picked fruit (Grossbeckler 1989, pers. comm.).

We present phenological data from trees growing in Canberra, the southern-most region of distribution, as well as observations from Atherton in north Queensland. Results of controlled-temperature studies on germination are also presented and discussed in relation to nursery practices.

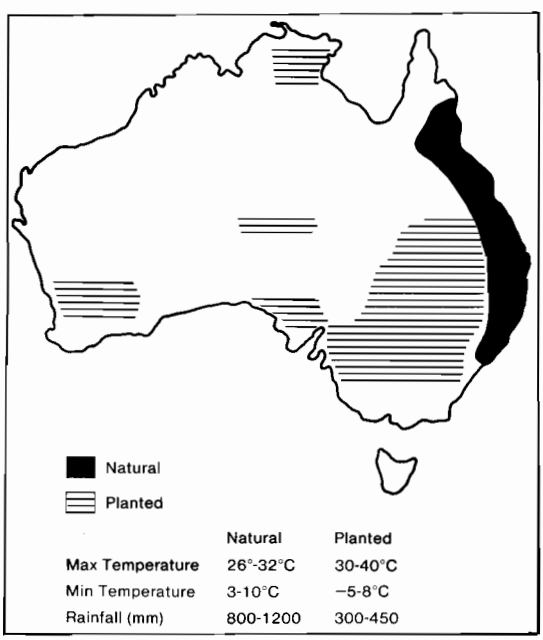

Fig. 2. *Melia azedarach* distribution in Australia.

Methods

Phenological Studies

Phenological observations were made on 22 10-year-old trees growing as ornamentals at Black Mountain, Canberra (lat. 35° 10′S; long. 140° 4′E; alt. 600 m). All trees were obtained via the nursery trade and the seed origin was subtropical New South Wales. Phases of development recorded were: bud burst; flowering; shoot elongation; fruit growth; new flower bud initiation; leaf yellowing; leaf drop and fruit drop. At two weekly intervals fruit was collected at random from all trees,

bulked, and 100 fruit were measured for length, width and dry weight. Fifteen panicles were tagged on four trees and flower and fruit numbers recorded at regular intervals.

Time of flowering, leaf drop and fruit drop were also made on trees growing in naturally regenerated stands 5 km west of Atherton (lat. 17° 16′S; long. 145° 29′E; alt. 753 m).

Seed Germination

Three experiments were undertaken to investigate the influence of time of harvest, storage, pretreatment and temperature on germination.

Experiment 1 Fruit was randomly sampled from all trees, at six different dates (Table 1), planted (50 fruit × 4 replicates) in pots of soil to a depth of 2.5 cm and kept continually moist. Pots were placed in a glasshouse with air temperature controlled to 35°C day and 25°C night.

Table 1. Radicle emergence from a range of sample dates.

Sample date	Radicles emerged*	Days to first emergence
18/7	40	68
30/7	26	42
13/8	24	39
10/9	32	56
24/9	34	42
05/11	4	14

*Out of 50 radicles. Test completed 30/12.

Experiment 2 Fruit was collected in August when the fruit was orange/yellow, flesh removed, and endocarps stored at 3°C for six months in sealed tins. Endocarps were planted in trays filled with 50:50 perlite and vermiculite to a depth of 2.5 cm. One tray containing 25 endocarps × 3 replicates was placed in each of 7 glasshouses, in the CSIRO Phytotron, Canberra, running at day/night temperature regimes of 35/30; 30/25; 27/22; 24/19; 21/16; 18/13 or 15/10°C. Day length was extended to 16 hours by incandescent lights. For one replication (25 endocarps) the endocarp was cracked to improve water penetration. Trays were watered twice a day and three times in the hotter regimes.

Experiment 3 Fruit was collected in August, flesh removed, and endocarps stored at 3°C for 6 months. Immediately prior to the germination test, 100 individual seeds were removed from randomly selected endocarps taking care to retain only those which were not damaged. Seeds were then soaked in water at room temperature for 24 hours before sowing. Twenty five seeds were placed in 9 cm glass petrie dishes containing vermiculite (7g) moistened with 30 ml of distilled water. Four replicates were placed in germination cabinets running at a constant temperature of 20, 25, and 30°C respectively and at an alternating day/night regime of 30/20°C. All had 12 hours of light per day and seeds were watered when necessary.

Results

Phenology

The timing and duration of developmental stages of trees growing in Canberra and Atherton are presented in Fig. 3. The complete cycle from

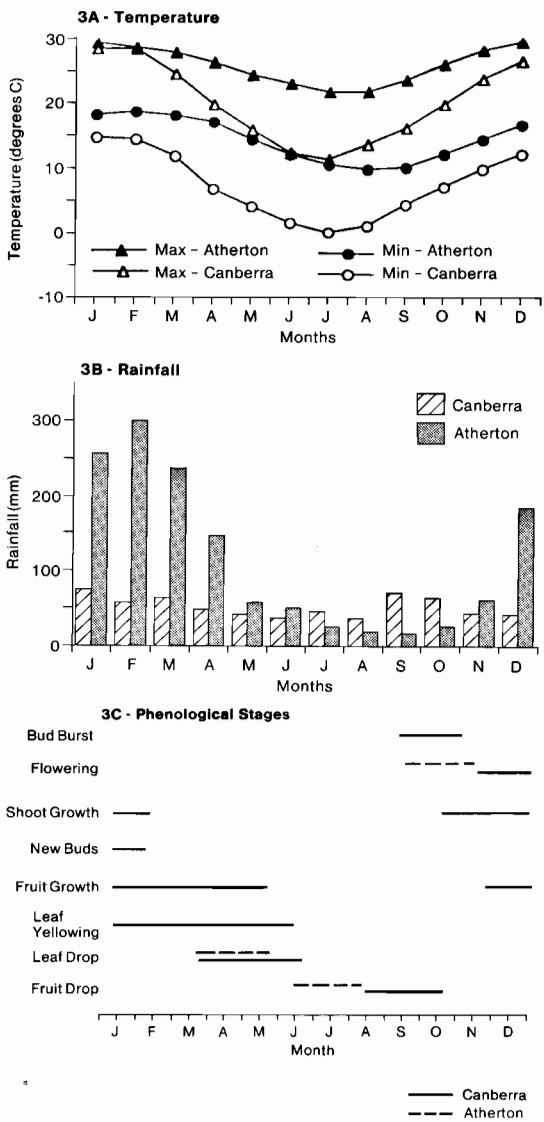

Fig. 3. Phenological patterns of *Melia azedarach* grown at Atherton, Queensland and Canberra, ACT.

bud burst to fruit drop took 10 months at Atherton and 12 months at Canberra. Maximum and minimum temperatures and rainfall are also presented.

Buds emerged from the axils of leaf (petiole) scars formed during the previous season's growth. Green leaf tissue became visible in October followed by the blue/mauve flower buds. The large loose panicles, 20 cm in length, contained up to 120 flowers. Individual flowers consist of five sepals, five petals, a staminal tube of 10–12 stamens with minute anthers on the end of long filaments. The ovary contains five locules each with a single ovule. Flowering occurred over a period of 3–4 weeks and the start and duration were fairly constant over 4 years (1983–86) of observations. First flower at Canberra was observed between 11 and 19 November and flowering finished between 10 and 19 of December. Shoot elongation commenced in October and increased to a maximum by early January when leaf development ceased.

Fruit dry weight at Canberra increased through to May then declined slightly before abscissing in October (Fig. 4). Fruit length and width increased up to May then declined as the flesh became soft and later dried (Fig.4). Fruit was initially a shiny green slowly turning orange/yellow by total leaf fall in June. There were initially 27.2 ± 19.3 fruit per panicle but only 8.0 ± 2.7 remained at maturity.

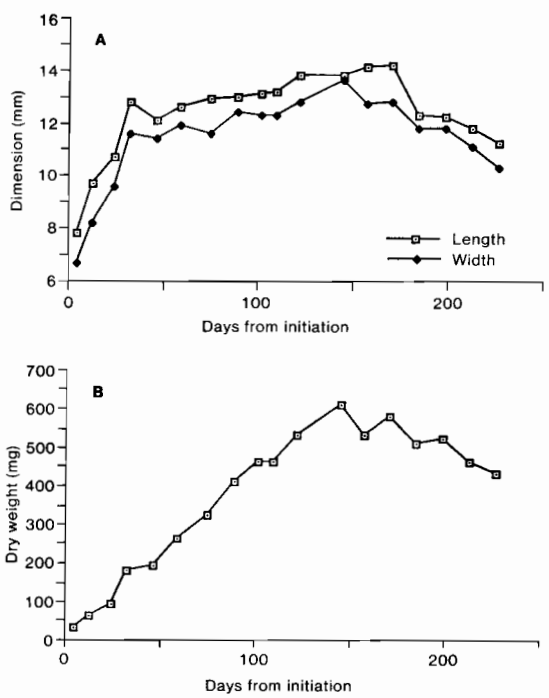

Fig. 4. Variation in fruit length, width and dry weight.

Leaf yellowing was first observed in late January commencing with those inside the canopy. In most years leaf fall commenced in March with all leaves shedding by late June.

The attractive orange fruit remained on the tree over winter. Panicles abscissed in large numbers in July at Atherton, while at Canberra this did not occur until October, at the time of bud burst and new leaf emergence.

Seed Germination

Experiment 1 Germination was recorded in endocarps from all sample dates though greatly reduced in the November sample (Table 1). A small sample of fruit collected in September was sown with the flesh removed. These endocarps had better germination and their radicles emerged earlier than from those fruit with their flesh intact.

Experiment 2 There was a marked decline in germination at temperatures below 35/30°C. Forty-seven out of 75 radicles had emerged at 35/30°C after 67 days, compared to 2 at 30/25°C and 27/22°C and 6 at 24/19°C. No emergence was recorded at temperatures below 24/19°C. However, fruit from the lower temperature treatments were transferred to 35/30°C after 50 days, and eight had emerged after a further fifteen days.

Experiment 3 After 50 days 28% emergence was recorded at 30°C, with only 4% at the lower regimes. Similar results were found on a thermogradient bar study with emergence occurring only at a constant 30°C (Gunn unpubl., 1988).

Discussion

In Canberra ripe fruit can be harvested directly from the tree, for up to 5 months (Table 1) before it falls in the spring when new growth commences. At Atherton fruit remains on the tree for a shorter period before falling when moisture levels decline in July. By contrast in Kenya seeds of *M. volkensii* mature in 11–13 months but phases of fruit development lack a seasonal pattern (Milimo and Hellum 1987).

Our results indicate that seed viability may be reduced over time, as fruit sampled in November had considerably lower germination rates than from earlier samples. However temperatures had increased by this sampling and flesh on the seed coat had dried hard. Also, the seed coat was now considerably harder than at earlier samplings and water penetration would have been restricted. It is not clear when seed is first viable, as fruit recently collected in early June has germinated.

Maximum seed dry weight had occurred by May/June (Fig. 4) when the fruit was still green,

Table 2. Number of ovules per endocarp from three locations.

Location	Seedlot*	Number of endocarps	Mean ovules/endocarp	Mean weight endocarps (g)
Atherton	14500	26	3.65 ± 1.35	0.608 ± 0.139
Mt Garnet	14501	36	2.07 ± 0.98	0.264 ± 0.072
Canberra	N.A.	10	3.30 ± 1.34	0.342 ± 0.104

*CSIRO Seed Centre seedlot number.

thus making it difficult to recommend a visual indicator for maturity. However once the fruit turns orange/yellow the seed is viable. Removal of the flesh improved germination, suggesting the presence of an inhibitor. This could be an adaptation for dispersal so that the parent is not surrounded by offspring when the seed has fallen. The fact that trees are reported growing on some Queensland islands suggests that birds are seed dispersal agents. Dispersal by water is unlikely.

Each endocarp has the potential to produce five viable ovules. Our results indicate that this potential was not achieved at three locations (Table 2). Further sampling from a wide range of locations is required to see if variation in ovule number is related to environmental conditions or is a genetic limitation. The use of x-ray techniques for screening seed samples for ovule numbers needs further study.

The failure to germinate at temperatures below 24°C may explain the lack of seedlings under trees in Canberra. Even though the ground had been cultivated and the seeds buried, no seedlings emerged. Our results show that high temperatures (about 30°C) are required for good germination. Storage or growth at lower temperatures had little effect on germination even under high moisture conditions. This is not surprising, as *Melia* is a plant of the tropics and subtropics.

A number of references (Everist 1974; Turnbull 1986; Hyland pers. comm.) contend that *Melia* can be readily propagated by seed even after several years. More precise studies are required to determine time of seed maturation and optimum time of collecting, pretreatment methods, storage requirements and seed longevity.

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The Influence of Temperature on Germination of *Melia volkensii* Seeds

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Abstract

The influence of alternate and constant day and night temperatures on germination of *Melia volkensii* Gurke seeds was examined. Total germination and germinative rates were significantly different for the two temperature regimes. The optimum temperature was the same for both constant and alternate temperatures. Seeds failed to germinate in temperatures above 37°C. Therefore, in the semi-arid areas where the species grow naturally, shading of seedbeds is recommended as a measure of lowering nursery soil temperatures.

SEED of *Melia volkensii* Gurke, are dormant and this constitutes a problem for nursery personnel (Dale and Greenway 1961). In order to determine the type of dormancy and how to overcome it, various germination studies were conducted (Milimo and Hellum 1989). Germination depends on certain environmental factors such as light, temperature and aeration, therefore a first step in this study was to ascertain the optimum levels of environmental conditions suitable for seed germination in the species. This paper reports results of an experiment on the influence of alternate and constant day and night temperature regimes on *M. volkensii* seed germination.

Germination of seeds is the initial, and under some circumstances, critical step in afforestation by natural or artificial means. Therefore, a fundamental knowledge of germination ecology is essential to forestry. Also, forestry research workers will find it difficult to undertake experiments successfully if the germination requirements of the species under study are a matter of conjecture (Ackerman and Farrar 1965).

Methods

Extracted seeds were x-rayed on Kodak X-Omat TL film (Health Science Markets Division, N.Y.), at 15 Kv, 5 mA for 30 seconds in an X-raying chamber (Model M110 NH, TFI Corp-CT), at a distance of about 47 cm from the energy source. By examining the x-ray negatives, seeds with incomplete cotyledons, undeveloped embryos and internally damaged embryos were excluded during computation of results (Muller-Olsen et al. 1965). Seeds were then soaked in water for 18 hours and cut longitudinally at the micropylar end.

Cut seeds were laid to germinate on moist Kimpak, in transparent polystyrene germination boxes (Wang and Ackerman 1983) in Conviron growth chambers (Model G30). The five alternate and constant temperatures tested were: 25°C days and 22°C nights, 27°C, 32°C, 37°C, and 42°C days and 25°C nights; and 25°C, 27°C, 32°C, 37°C and 42°C ± 1°C days and nights. Days and nights were 12 hours long.

Results

Germination commenced in 3–4 days and was largely complete in 14 days. Mean per cent total germination under alternate day/night temperatures differed significantly ($P \leq 0.01$), with the lowest value 1% for 42°C and 25°C and the highest 78% for 32°C and 25°C (Table 1). Total germination under constant temperature regimes also differed significantly ($P \leq 0.01$). The highest value was observed for 32°C (83.16%) and no germination for 42°C day and night (Table 1).

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Table 1. Mean per cent total germination and germinative rates for alternate and constant day and night temperatures (n =)

Temperature (°C)	Total germination (%)		Germinative rates (days)	
A: Alternate temperatures				
25/22	59.94 ± 12.36	b	9.65 ± 0.25	a
27/25	60.36 ± 16.03	b	9.31 ± 0.09	a
32/25	76.46 ± 14.52	b	7.48 ± 0.42	b
37/25	54.71 ± 9.13	b	5.23 ± 0.17	c
42/25	1.05 ± 2.10	a	1.15 ± 2.30	d
B: Constant temperatures				
25/25	55.95 ± 16.99	c	6.58 ± 1.52	a
27/27	61.04 ± 6.35	b,c	5.35 ± 0.70	a,b
32/32	83.16 ± 13.12	a	4.26 ± 0.58	b
37/37	76.94 ± 14.46	a,b	4.21 ± 0.27	b
42/42	0.00 ± 0.00	d	0.00 ± 0.00	c

Within each column, means followed by the same letter are not significantly different at $P \leq 0.05$ level. Note: days and nights were 12 hours each.

The relationship between day temperature and total germination is parabolic (Fig. 1), with the maxima at about 31°C ($Y = -525.40+38.74X -0.62X^2$, $R^2 = 0.82$) and 32°C ($Y = -754.41 +52.85X-0.83X^2$, $R^2 = 0.84$), respectively, for alternate and constant temperature regimes.

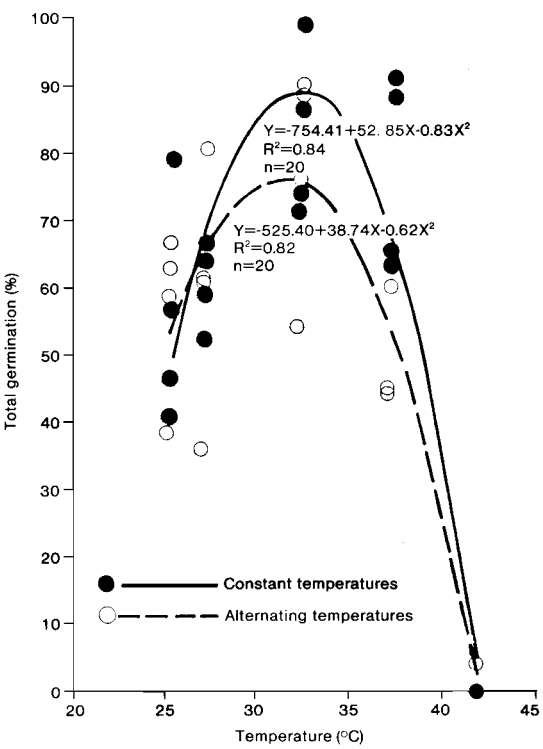

Fig. 1. Total germination (%) under alternating and constant day and night temperatures.

Germination rates also differed significantly among treatments for both alternate and constant temperatures ($P \leq 0.01$). The fastest germination rate (1.15 days) for alternate temperatures was observed at 42°C day and 25°C night while the slowest was at 25°C day and 22°C night. Note that total germination corresponding to this best germination rate is only 1%.

For constant temperatures, the most rapid germinative rate (4.2 days) was observed at 37°C and the slowest (6.5 days) at 25°C. Germinative rates for both temperature regimes increased with increase in temperature (Fig. 2).

The relationship between germinative rate and alternate temperatures is linear ($R^2 = 0.97$, $P \leq 0.01$), while that of constant temperatures is curvilinear ($R^2 = 0.60$, $P \leq 0.01$; Fig. 2). Since seeds failed to germinate or germinated poorly at temperatures above 37°C, germinative rate values for 42°C were excluded during the computation of these relationships (Fig. 2).

Discussion

The seed population behaved fairly homogeneously as far as germination capacity was concerned, for most seeds succeeded in germinating at some time or another at all temperatures from 7 to 37°C. However, the population was heterogeneous with respect to germination rate. The biological significance of this may be that a high potential germinability is combined with temporal spread, even when the temperature conditions are optimal (Bewley and Black 1985).

The optimum temperature was the same for total germination and germinative rate for both

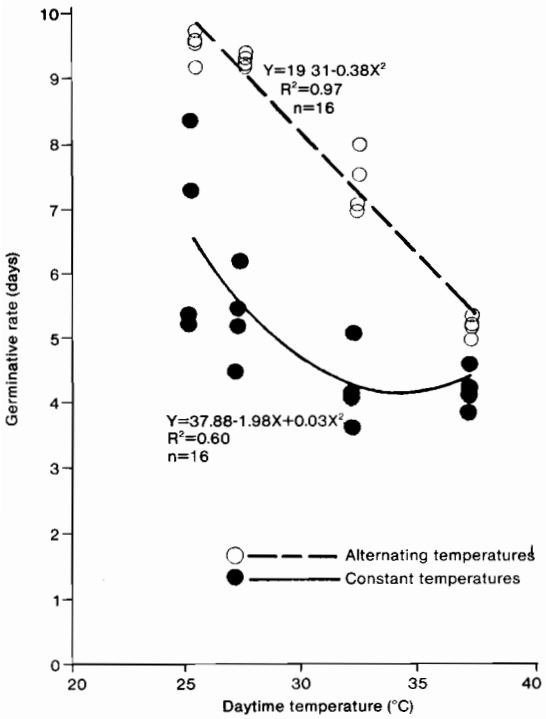

Fig. 2. Germinative rates under different alternating and constant day and night temperatures. (In alternating regimes nights were 25°C, except for 25°C day which had 22°C night.)

temperature regimes. Although germinative rates for higher temperatures above the optimum are better, the corresponding total germination was very poor for temperature regimes above 37°C (Table 1). Results of this study indicate that seeds of *M. volkensii* when scarified to break their dormancy are capable of germinating between 25 and 37°C. Extrapolation of curves in Fig. 1 indicate that germination slightly below 25°C is possible. This may suggest that temperature is not a critical factor during germination of pretreated seeds under ambient conditions in its natural range. The fact that total germination at 42°C for both temperature regimes was very poor may be an indication that imbibed seeds may not be able to tolerate exposure to soil temperatures of this magnitude, even for short periods (Table 1). This suggests that great care ought to be exercised in nurseries to avoid exposure of imbibed seeds to temperatures below 25°C and above 37°C.

The natural distribution range for *M. volkensii* is semi-humid to semi-arid and arid. The seasonal temperature changes in this zone are relatively small, and closely parallel the seasonal changes in rainfall. High temperatures (mean maximum of 32–40°C) occur at times of high rainfall, and vice versa (Fenner 1982). Corresponding soil (sandy soils) temperatures would be even higher. Therefore, going by results presented in this paper, attempts to germinate seed in unshaded seedbeds may lead to germination failure. As a result, it is strongly recommended that seedbeds be shaded and watering only done very early in the morning and late in the evening when soil temperatures are low. However, the effect of temperature also interacts strongly with other factors such as water stress and light (Koller 1972). Therefore, studies leading to a clear understanding of these interactions are recommended.

Germination rates were fastest for seeds incubated in 42/25°C and 42/42°C. Germination of nondormant seeds can occur over a temperature range within which there is an optimum, at which the highest percentage is obtained in the shortest time (Kramer and Kozlowski 1979; Gordon 1972). However, results of this study are contradictory to this view, since total germination corresponding to the fastest germination rates was also the lowest. This relationship was exhibited in another study where seeds were incubated, increasing oxygen concentrations (Milimo and Hellum 1989). An explanation for the relationship is currently not known.

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Effects of Seed Pretreatments on the Development of *Acacia auriculiformis* and *A. holosericea* Seedlings

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Abstract

Acacia auriculiformis and *A. holosericea* seeds were nicked, immersed in hot water and soaked in sulphuric acid to improve germination and to determine the effects of the various pretreatments on germination and vigour of subsequent seedling growth. Nicking achieved the highest germination % and germination energy for both species, but the vigour of seedling growth was lower when compared to other treatments. For *A. auriculiformis* soaking seeds in acid for 30 min and immersing seed in water at 95°C for 5 min produced high germination and energy and most of the resultant seedlings were in the high vigour category. For *A. holosericea.* hot water (1 and 5 min) produced a high germination percentage and the seedlings showed high vigour of growth. Acid and hot water treated seeds exhibited more surface fungal infection than nicked seeds.

SEEDS of *Acacia* spp. have hard seedcoats that completely prevent the imbibition of water and exchange of gas, thus preventing initiation of the germination process. Various scarification methods like soaking in commercial grade sulphuric acid and nicking at the micropylar end have been reported to enhance germination of most African acacias (Doran et al. 1983; Kariuki 1987; Ngulube and Chipompha 1987; Sniezko and Gwaze 1987). Germination of certain Australian acacias was enhanced by immersing the seeds for 1–5 min in boiling water (Doran and Gunn 1986). Sulphuric acid has been reported to be equally effective in improving the germination of some Australian acacia seed (Doran et al. 1983, Kariuki 1987, Acoba 1987).

Seed scientists have managed to break the seedcoat of most hard seeds and enhanced germination. No work has been done to look at the effects of the pretreatments on germination energy and development of the subsequent seedlings. Observations in the Regional Seed Centre seed testing laboratory in Harare showed that acacia seeds scarified using severe pretreatments exhibited more seed-borne surface mycoflora than nicked seeds.

It is becoming increasingly obvious that many lesser known species (including indigenous species) require specialised nursery techniques to obtain maximum recoveries from seed and emphasis needs to be given to nursery work if seed potentials are to be fully realised. Nursery records at the Forest Research Centre (FRC) from 1986 to 1988 show that nursery recoveries of most acacias are very low. The causes of poor germination were identified as: (a) incomplete scarification; (b) over scarification resulting in 'cooked' seeds; (c) damping off; (d) seedlings of poor vigour. There is a need to do research to come up with a nursery technique that covers pretreatments, sowing and transplanting times.

This paper looks not only at germination, but the vigour of germination and seedling development and other effects of the various pretreatments. A long-term study of the performance of seedlings raised from treated seeds is also necessary and this can be done by selecting seedlings from vigour classes and observing growth in pots.

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Materials and Methods

Seeds of two Australian *Acacia* spp. that are promising in afforestation projects were used. *A. auriculiformis* seed (Stock No. 17450) was collected at Coopers Creek, Northern Territory, in 1988. *A. holosericea* (10927) seed was collected at Helenvale, Queensland in 1983. Seeds of uniform size were selected from the working sample to reduce non-treatment variation, as vigour was found to be positively correlated with seed size (Niembro et al. 1978, Bonner 1987).

Five hundred seeds of each species were divided into 5 subsamples of 100 seeds. Each subsample was further divided into 4 replications of 25 seeds. Five treatments: nicking (Doran et al. 1983), water immersion for 1 and 5 min at 95°C (Doran et al. 1983; Acoba 1987; Kariuki 1987) and concentrated sulphuric acid soaking for 15 and 30 min (Doran and Gunn 1986, Acoba 1987) were applied to each species.

The control (nicking) and the treated seeds were placed on moist blotting paper supported on glass plates as described by Dasgupta et al. (1976). A completely randomised design was used. The treatments and replications were randomly arranged in one water bath tank. The tank was set to run at 20°C at night (16 hrs) and 30°C during the day (8 hrs). Light during the day was intensified by using cool white fluorescent tubes. The number of seeds germinated and the height of seedlings was recorded at 3-day intervals. The seedlings were grouped into vigour categories based on germination and seedling height. The final records were taken after 27 days.

The index of vigour of germination was estimated by calculating the germination energy which is the germination percentage at maximum daily germination speed (Seward 1980). Morphological appearances and fungal surface infection were noted.

Results and Discussion

Germination and Germination Energy

Table 1 shows germination percentage achieved after 27 days and germination energies at 9-day energy period. Nicking achieved the highest germination percentage for both species. Hot water treatments produced good results. For both species soaking seeds in acid for 30 min resulted in higher germination percentages than soaking seeds in acid for 15 min (see Table 1). The latter was not severe enough to render the seedcoat permeable to water.

The final germination percentage showed only the proportion of seeds reaching the emergent stage but did not reveal the time taken to reach the stage. Germination energy provided the information on the distribution of germination.

Nicking, hot water (5 min) and acid (30 min) scarification produced high energies of germination for both species with most of the seeds germinating in the first 9 days (see Table 1). The pretreatments if applied in the nursery may produce uniform planting stock resulting in high nursery recovery. The less severe pretreatments (hot water 1 min and acid 15 min) produced low energies of germination after 9 days when compared to the more severe treatments (hot water 5 min and acid 30 min). In the nursery such treatments produce seedlings of various sizes resulting in poor nursery recovery.

Seedling Vigour

Vigour categories achieved by the pretreatments are shown in Table 2. It was interesting to note that although nicking produced the highest germination percentages and energies for both species most of the resultant seedlings were in the low vigour class (see Tables 1 and 2). For *A. auriculiformis* immersing seed in hot water (5 min)

Table 1. Germination percentages after 27 days and germination energies after 8 days of *A. auriculiformis* and *A. holosericea* seeds that were pretreated.

Treatments	*A. auriculiformis* Germ. %	Germ. en.	*A. holosericea* Germ. %	Germ. en.
Nicking	100	98	97	97
Hot water 1 min	76	65	93	83
Hot water 5 min	85	75	90	90
Acid 15 min	50	35	30	27
Acid 30 min	95	80	80	77

Germ. % = germination percentage
Germ. en. = germination energy

Table 2. Vigour categories of *A. auriculiformis* and *A. holosericea* seedlings based on germination and height after 27 days expressed as percentages.

Treatments	*A. auriculiforms*			*A. holosericea*		
	NG	LV	HV	NG	LV	HV
Nicking	0	60	40	3	77	20
Hot water 1 min	24	16	60	7	20	73
Hot water 5 min	15	15	70	10	27	63
Acid 15 min	50	13	37	70	3	27
Acid 30 min	5	2	93	20	7	73

NG = non-germinated seeds
LV = low vigour seedlings below experimental mean height
HV = high vigour seedlings above experimental mean height
Exp. mean height for *A. auriculiforms* = 6.557 cm after 27 days
Exp. mean height for *A. holosericea* = 7.365 cm after 27 days

resulted in more seedlings in the high vigour category than the 1 min treatment. The 1 min treatment produced more high-vigour seedlings than the 5 min treatment for *A. holosericea*. Soaking seeds in acid for 30 min produced more high-vigour seedlings than the other treatment for both species.

An analysis of variance for a complete randomised design was done on the height data for seedlings raised from treated seeds to test for treatment effect differences.

For *A. auriculiformis* the treatments effect was associated with an F-ratio of 26.38 (>3.06 table value) suggesting treatment differences at 5% level of significance. A comparison of treatment mean heights was performed using the Least Significance Difference (LSD) multiple range test (see Table 3). The comparison of treatment means showed that immersing seed in hot water (5 min) and soaking seeds in acid (30 min) resulted in seedlings of higher vigour than all other treatments. Although nicking produced a high germination percentage and energy (see Table 1) the resultant seedlings were of low vigour of growth when compared to the hot water (5 min) and acid (30 min) treatments. A comparison of the hot water treatments indicated that although the germination percentages were almost equal, the mean heights of the seedlings were significantly different at 5% level (see Table 3).

Table 3. Comparison of treatment mean heights for *A. auriculiforms* seedlings raised from treated seeds.

Treatment	Ranked means	LSD(H) + mean
Acid 15 min	3.535 a	4.85
Hot water 1 min	5.815 ab	7.13
Nicking	6.255 bc	7.57
Hot water 5 min	7.575 cd	8.89
Acid 30 min	9.603 d	

Means followed by the same letter are not significantly different at the 5% level. (LSD multiple range test)

The treatments effect for *A. holosericea* was significant at the 5% level of significance (F-ratio was 7.78). Treatment means were compared using the LSD multiple range test (Table 4). Immersing seeds in hot water (1 and 5 min) and soaking seeds in acid (30 min) produced seedlings of equal height. The treatments achieved lower germination percentages and energies than nicking (see Table 1), but the vigour of growth of the subsequent seedlings was higher. Soaking seed in acid for 15 min produced seedlings with the lowest mean height after 27 days.

Table 4. Comparison of treatment mean heights for *A. holosericea* seedlings raised from treated seeds.

Treatments	Ranked means	LSD(H) + mean
Acid 15 min	4.705 a	6.770
Nicking	6.260 ab	8.325
Acid 30 min	7.860 bc	9.925
Hot water 5 min	9.405 c	
Acid 30 min	9.595 c	

Means followed by the same letter not significantly different at the 5% level. (LSD multiple range test)

For both species nicking produced the highest germination percentage and high energy of germination, but most of the resultant seedlings were in the low vigour category. This could have been caused by physical restriction of the expansion of the embryonic parts by the remaining seedcoat and the unrestricted inflow of water into cells close to the nicked part causing shearing and tearing of hydrated cells from dry ones. This could have led to physiological stresses causing poor growth after germination.

Immersing seed in hot water may have enhanced germination by providing a stimulatory

effect on the germination process and by causing the lens tissue to rupture creating a passage through which water entered into the seeds (Cavanagh 1987). Sulphuric acid rendered the seedcoat soft causing uniform inflow of water and unrestricted expansion of embryonic parts.

Germinating seeds and seedlings from seeds immersed in hot water and soaked in acid exhibited more surface mycoflora than those nicked for the two species. Possible reasons could have been that the severe treatments removed the protection offered by the coat resulting in metabolites leaking out providing a nutritious medium for fungal growth. The treatments could have enhanced the germination of the fungal spores.

Conclusions

The pretreatments had different effects on germination and vigour of seedling growth. For *A. auriculiformis*, soaking seed in sulphuric acid for 30 min produced the best results and for *A. holosericea*, the best pretreatment was immersion in hot water for 5 min. The ideal pretreatment for seeds with seedcoat dormancy is one that results in high germination percentage and energy while producing seedlings of high vigour. The severe treatments encouraged growth of surface-borne fungi and this can cause damping-off in the nursery. A pilot test should always be conducted to determine the best pretreatment that ensures maximum nursery recovery of high quality seedlings.

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A Study of the Causes of Poor Germination of *Anogeissus leiocarpus* Seeds

L.M. Some, C.S. Gamene, and H. Verwey*

Abstract

Production of plants of *Anogeissus leiocarpus* from seeds, the only method used so far in several countries, remains a difficult process because of the low germination rate registered in nurseries as well as in laboratories. To explain the reasons for the poor germinability of these seeds, four methods have been used: germination tests under controlled temperature conditions; comparative pretreatments methods; dissection of fruits; X-ray radiography of fruits. These methods have been used to determine that the low germination rates are mainly due to the low fertility rate of fruits (5% on average). An increase of the success rate in nurseries would therefore imply an increase of the fertility of the fruit batches used, or that we should resort to vegetative reproduction.

THE good planning and the profitability of production of plants in forest nurseries demands the mastering of techniques to speed up germination and obtain a more reliable percentage germination of seeds sown.

Since its creation, the Centre National de Semences Forestières (CNSF) — National Forest Seed Centre — of Burkina Faso has worked to improve the techniques formerly used; all the comparative pretreatment tests carried out have been done with the desire to improve these unsatisfactory techniques. After three years of research some species which are either already important in the forestry policy of Burkina Faso, or which are to be promoted (including *Anogeissus leiocarpus*) have remained unresponsive to all the pretreatment methods used.

What is *Anogeissus leiocarpus?*

Anogeissus leiocarpus (syn. *Anogeissus schimperi*, Hochst ex Hutch and Dalz; *Conocarpus leiocarpus* D.C.) belongs to the family Combretaceae. It is a species found on wet soils, especially around ponds, in river valleys and forest galleries. The tree can reach 30 m and produces excellent firewood and charcoal. The leaves are a source of yellow dye; green or dry, they make fodder for cattle, sheep and goats. This type of tree is found in the zone of Africa between isohyet 200 mm and the humid tropical forest from Senegal to Sudan and Ethiopia and to the South as far as Zaire, (Maydell 1986). The yellow or brown fruits are small (3 mm) with two wings.

A. leiocarpus is an important forest component of the sparse forest region of Burkina Faso. In June 1986, five seedlots representing 56 kg (CNSF 1987) were available at CNSF. Until now this tree has not occupied a very important part in the afforestation program, partly because of the difficulties of production in nurseries; very low germinating rates (1–2%) were obtained each time (Table 1).

Research Methods

Four methods have been used to investigate poor germinability: germination tests under controlled temperature conditions; comparative pretreatment tests; dissection of fruits; X-ray radiography.

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Table 1. Summary of germination tests of *Anogeissus leiocarpus* seeds.

Lot	Dates of harvest	Dates of tests	Pretreatments applied	Germination after 28 days (%)
217	5–12–84	16–1–85	No treatment	0
			Soaking in water for 24 hours (SW 24 h)	2
			Boiling + SW 12 h	0
			Cooking + SW 12 h	0
281	31–12–84	24–1–85	No pretreatment	0
		24–1–85	No pretreatment	0
285	8– 1–85	7–2–85	SW 24 h with renewal of water 24 h	0
				2
		9–3–85	SW 24 h	0

Germination Tests under Controlled Conditions

The main aim was to speed up germination under varied temperature conditions. The tests were carried out in an incubator with four replicates, each replicate containing 50 seeds i.e. 200 seeds per treatment. The seeds were scarified by hand using a nail-cutter. A range of temperatures was tested: 10, 20, 25, 28, 30, 35°C; each temperature corresponded to one treatment. Whereas the program temperatures remain constant, a regime of 10 hours of darkness and 14 hours of white light was used. The sowing was done on filter paper soaked with 5 ml distilled water.

There was no germination after 10 days at any of the temperatures used. The germination of *A. leiocarpus* seeds is thus not stimulated by any of the temperatures used, and it is not possible to explain their germination by a negative influence of an excess or a lack of heat.

Comparative Pretreatment Tests

For the treatment, we used four replicates of 50 seeds each. Fourteen pretreatments were compared to a control (no treatment). These pretreatments were:

Temperature pretreatments

- Boiling: Water was brought to the boiling point, the source of heat was cut off and the seeds put to soak in this water for 24 or 48 hours before being sown.
- Cooking: Once the water was brought to boil, the seeds were added and the source of heat maintained for 1–10 minutes. The seeds remained in this water for 24 hours.

Scarification

- Mechanical scarification: This was done by hand. A notch was made on the skin of the fruit with a nail-cutter.
- Chemical scarification: The seeds were soaked in 98% sulphuric acid (H_2SO_4) for various periods: 1, 5, 10, 30, 60 minutes. The seeds were stirred and at the end of the set period, they were rinsed three times in tap water then left to soak in tap water for 24 hours before being sown.

Simple soaking in water

- The seeds were soaked in tap water for varying periods: 24, 48, 72 hours, at the end of which they were rinsed in tap water then sown.

Sowing was done in germinating tins with untreated river sand used as substrate. A volume of 446 cm^3 of sieved sand was used per tin. It was moistened with 80 ml of distilled water. The seed beds were made with the help of studded planks. The seeds were placed on the beds (1 seed per bed) and covered with about 1 cm of sand. Germination counts were taken every two days for 28 days. The tests were carried out under prevailing laboratory conditions.

Table 2 shows the arithmetic average of the germination percentage obtained for the four replicates of each treatment. The Hartley tests makes it possible to say that the variances are homogeneous for the 6 pretreatments for which we have noted some germination. We can therefore analyse the variances and reach the conclusion that there is no difference, either between replicates or between treatments.

Although soaking in H_2SO_4 for 1 minute then soaking in water for 24 hours gives the highest rate of germination (3%), it can be noted that this rate is low and that the poor germination is in fact not linked to the poor method of removing the tegumentary obstacle caused by the waxy texture of the fruit on the seed. This is even more true as the soaking in H_2SO_4 for 5, 10, 30 and 60 minutes had destroyed all the tissues, giving a brownish mixture in which it was impossible to distinguish any seed or fruit tissues.

Dissection of fruits

The fruits were dissected by cutting them lengthwise under a magnifying glass. The dissec-

Table 2. Summary of the germination percentage

Pretreatment	Germination %
• None	0.5
• Soaking in water for 24 hours	0
• Soaking in water for 48 hours	1
• Boiling + Soaking in water for 24 hours	0
• Boiling + Soaking in water for 48 hours	0
• Cooking 1 minute + Soaking in water for 24 hours	0
• Cooking 5 minutes + Soaking in water for 24 hours	0
• Cooking 10 minutes + Soaking in water for 24 hours	0
• Soaking in H_2SO_4 for 1 minute + Soaking in water for 24 hours	3
• Soaking in H_2SO_4 for 5 minutes + Soaking in water for 24 hours	0
• Soaking in H_2SO_4 for 10 minutes + Soaking in water for 24 hours	0
• Soaking in H_2SO_4 for 30 minutes + Soaking in water for 24 hours	0
• Soaking in H_2SO_4 for 60 minutes + Soaking in water for 24 hours	1.5
• Soaking in water for 72 hours	0.5
• Scarification	0.5

tion was repeated 4 times with 50 fruits in each replication. The internal structure of the fruits was observed and described.

The results of the dissection were: (a) fertile fruits with internal cavity containing a seed — 5.5%; (b) infertile fruits with internal cavity with no seed — 6.5%; (c) infertile fruits with no internal cavity—88%.

(a) Fruits with an internal cavity and containing seeds. These fruits were considered fertile. The ovule was able to develop to the end.

(b) Fruits with internal cavity but containing no seed. These were considered infertile. Sometimes there was an imperfectly developed ovule. The development of the embryo had been stopped at some stage of its growth.

(c) Fruits without any internal cavity and therefore containing no seed. These fruits are infertile and represent almost the total of all the fruits (88%). It could be considered that the development process of the fruit had not even begun.

X-ray radiography

This is a very reliable tool for studying the anatomy and quality of forestry and agricultural seeds (Kamra 1961, 1973). Four seedlots were used, No 12 CNSF, No 281 CNSF, No 285 CNSF, No 585 CNSF, and for each lot only one replicate of 5 × 5 seeds (25 seeds). No treatment was given to the seeds.

The machine used for the radiography is FKA — 2; the films were Structurix D7, (Kamra 1975), made by Agfa Gevaert, Antwerp (Belgium). The parameters taken into consideration were a voltage of 14000 V (14 KV), and amperage of 5 A with exposure time of 6.5 seconds and a focus distance picture area of 50 cm. The films were treated as follows: developed in Structurix, Developer G. 127 and fixed in Universal Fixative G. 321 made by the same firm as the films.

Two groups of fruits were observed, fertile and infertile. Their percentages according to the lots are given in Table 3.

Table 3. Summary of the fertility of fruits as revealed through x-ray radiography

Groups of fruits	Fruitlot No. 1	2	3	4
Fertile fruits				
• Showing a central cavity with a developed seed	4	0	8	16
Infertile fruits	96	100	92	84
• Showing a central cavity filled by mass	40	0	4	0
• Showing undeveloped ovules	0	4	28	28
• Showing neither central cavity, nor seed	56	96	60	56

Fertile fruits Fruits showing a well developed seed occupying the central cavity of the fruit. The percentage of fertile fruits is 4% for the CNSF lot No 12, 0% for the CNSF lot No 281, 8% for the CNSF lot No 285 and 16% for CNSF lot No 565.

Infertile fruits (1) Fruits showing a central cavity filled by a mass of undetermined tissues; (2) fruits showing an undeveloped ovule in each half; (3) fruits showing no central cavity; the carpels have deteriorated.

Analysis

The results obtained through the various research methods led to the following preliminary conclusions:

— The low rate germination is not due to the impermeability of the fruit tissues only. When they are made permeable through mechanical or chemical scarification, the rate of germination does not increase (only 3% after soaking in acid for 1 minute).

— Accelerating germination through the use of several temperature regimes, a process that is usually effective for the germination of some seeds (Some and Kabore (1987) mentioned 28°C for *A. albida* seeds), and exposure to continuous white light did not improve the percentage of germination.

The low rate of germination could therefore be explained by this low fertility rate. Dissection and x-ray radiography show a fertility percentage of about 5%. By deducting the percentage of non-viable seeds and seeds which do not germinate due to ambient conditions (substrate, pretreatment, watering . . .) the seeds' actual germination percentage can be estimated at 2%. A germination rate of 5% was given according to the studies of IRBET/CTFT (1983). The amount of seeds needed to produce 100 trees of *Anogeissus leiocarpus* becomes very high (about 5000 fruits).

At this stage of research, we can speculate on the reasons for the low fertility rate. Two theories can be advanced:

- *Anogeissus leiocarpus* flowers in November-December, that is during the dry season, a period when the atmospheric moisture is very low; difficulties could arise on the germination of pollen grains and result in reduced fertilisation.
- *Anogeissus leiocarpus* flowers are hermaphrodite, but a big heterogeneity of sexual organs (producing pollen and ovules) could also lead to a low rate of fertile ovules.

It is therefore perhaps not possible to speak of speeding up germination but rather of increasing the fertility of fruits from a given lot. This fertility percentage could be increased either at the level of the mother-tree by improving the fertilisation of flowers or after the harvest.

In the first case (improvement of fertility), it will be necessary to study the biology of flowers and their fertilisation system. In the second case, the solution is to find a method to separate fertile fruits from infertile ones. This demands the development of an appropriate technique. Separation by density seems difficult because the difference in weight between the two categories of seeds appears to be very little, although we have not done any weighing to confirm this. In parallel with the fruit research, experiments of vegetative reproduction of this species could be carried out.

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Examining Seed Coat Characteristics with Energy Dispersive X-ray Spectroscopy

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Abstract

Energy dispersive x-ray spectroscopy (EDS) was used to examine seed coat characteristics in the genus *Pinus*. Seven species were sampled, viz., *P. clausa* (sand pine), *P. echinata* (shortleaf pine), *P. elliottii* (slash pine), *P. palustris* (longleaf pine), *P. strobus* (eastern white pine), *P. taeda* (loblolly pine), and *P. virginiana* (Virginia pine). EDS of both stratified and unstratified seeds revealed eight inorganic elements: Mg, Al, Si, P, S, K, Ca, and Fe. There were significant differences in elemental composition between stratified and unstratified seed coats of all species except sand pine, longleaf pine, and loblolly pine. Differences in elemental composition of seed coats may have application in identifying seed origins or in studies of the role of mobile elements during stratification and germination.

ENERGY dispersive x-ray spectroscopy (EDS) has been used to establish elemental composition of mature wheat seed (*Triticum aestivum* L.) by Mazzolini et al. (1985) and lodgepole pine (*Pinus contorta*) (El-Kassaby and McLean 1985). The use of EDS elemental signatures to distinguish samples within populations of diverse organisms has been widely demonstrated. Specifically, EDS has been used to identify stocks of salmon (*Oncorhynchus nerka* (Walbaum)) on the west coast of Canada (Calaprice 1971; Calaprice et al. 1973); origins of lesser snow geese (*Chen caerulescens* L.) (Kelsall and Burton 1977); ambrosia beetle (*Gnathotrichus suicatus* Le Conte) populations in a sawmill (McLean 1980); two field populations of western spruce budworm (*Choristoneura occidentalis* Freeman) (McLean et al. 1979); red turnip beetle (*Entomoscelis americana* Brown) populations in rape (*Brassica campestris* L.) fields (Turnock et al. 1980); and seeds from geographically isolated populations of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (El-Kassaby and McLean 1983).

Elemental energy spectra are reported here for seed coats of dormant and stratified seeds of seven southern pine species, and possible applications of this technique are suggested.

Materials and Methods

Seed coats of the following seven southern pine species were analysed for surface elemental signatures: sand pine (*Pinus clausa* Vasey ex Sarg.), shortleaf pine (*P. echinata* Mill.), slash pine (*P. elliottii* Engelm.), longleaf pine (*P. palustris* Mill.), eastern white pine (*P. strobus* L.), loblolly pine (*P. taeda* L.), and Virginia pine (*P. virginiana* Mill.).

All seven species were divided into two lots — unstratified and stratified seeds. Unstratified seeds were prepared as taken directly from storage (3°C), and stratified seeds were taken from storage then given standard pretreatment stratification (USDA, 1974). Each treatment consisted of 10 seeds of each species that were selected at random from seed lots.

The seeds were attached to opaque carbon planchets with small amounts of epoxy and placed in

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an AMR 1000A scanning electron microscope (SEM) equipped with a Kevex 5100 energy dispersive x-ray spectrometer. Seeds were coated with carbon using a Polaron Sputter Coater equipped with a carbon evaporation unit to minimise specimen charging. Data were collected with the following conditions being held constant: magnification, duration of probe (200 sec), accelerating potential (30 KV), tilt (45°), condenser lens setting (spot size 3), and working distance (12 mm). Spectra data were transmitted to a computerised smoothing routine followed by background subtraction. X-ray counts were recorded for each integrated elemental peak and total counts for the spectrum, after background subtraction. The elements detected were expressed as a percentage of total counts after background subtraction. Representative spectra were recorded photographically.

Elemental analyses were conducted relative to the same morphologic areas of all seeds, i.e. a flat area on the distal side of the longitudinal axis 2–3 mm above the micropylar end on the seed coat surface. Statistical analyses were carried out with the Bonferroni method of the multiple *t* test at $P > .05$. (Neter 1974).

Results and Discussion

EDS data show differences between stratified and unstratified treatments of pine seeds. Of the eight elements analysed (Mg, Al, Si, P, S, K, Ca, and Fe), only Ca and Fe concentrations showed no significant differences among species receiving the stratification treatment. Concentrations of Mg, Al, Si, S, Ca, and Fe showed no significant differences among species with unstratified seed treatments. This procedure does not distinguish inorganic from organic elemental concentrations for either S or P. When comparing the two conditions, all species except *P. clausa*, *P. palustris*, and *P. taeda* showed some seed coat differences for elements (Table 1).

There were elemental differences among species also (Fig. 1). For example, there are no significant elemental differences between *P. clausa* and *P. echinata*, but *P. clausa* and *P. elliottii* are significantly different for Mg, Si, P, S, and K. Stratified treatment shows elemental differences between species in nine combinations, with S appearing in seven, Si in six, P in six, Mg in four, K in four, and Al in one. For unstratified seeds, there are only three species combinations showing elemental differences, with P in one, K in one, and both P and K being significantly different between *P. strobus* and *P. virginiana* (Fig. 1). Again, the

	Pinus clausa	P. echinata	P. elliottii	P. palustris	P. strobus	P. taeda	P. virginiana
Pinus clausa			Mg, Si, P, S, K		Mg, Si	Al	
P. echinata			Si, P, S, K				
P. elliottii				P, S, K	P, S	Si, P, S	Mg, Si P, S, K
P. palustris					(K)		
P. strobus							Mg, Si, S (P) (K)
P. taeda							(P)
P. virginiana							

Fig. 1 Energy dispersive spectroscopy comparisons of elemental analyses from seed coats of stratified and unstratified seeds. Circled symbols represent elements present among species for the unstratified seed treatments.

Table 1. Energy dispersive spectroscopy comparisons of eight elemental analyses between seedcoats of stratified (s) and unstratified (u) seeds among seven *Pinus* species. Column values represent the percentage of x-ray counts in the total spectrum after background subtraction.

	Pinus clausa		*Pinus echinata*		*Pinus elliottii*		*Pinus palustris*		*Pinus strobus*		*Pinus taeda*		*Pinus virginiana*	
Element	s	u	s	u	s	u	s	u	s	u	s	u	s	u
Mg	1.1	1.0	1.2	1.1	3.9	1.9	1.2	2.0	3.0*	0.7*	1.1	1.0	0.8	1.7
Al	3.2	3.6	4.8	10.1	3.8	2.9	33.9	45.5	9.3	7.3	5.6	6.5	3.0*	10.6*
Si	70.9	49.9	68.8*	32.2*	10.8	49.6	48.5	22.0	31.9	37.7	57.2	40.5	77.2*	32.6*
P	2.0	3.5	2.3	4.3	26.4*	2.6*	2.3	2.4	9.6	1.4	3.1	1.3	1.7*	8.3*
S	4.2	2.5	4.6	3.8	25.9	19.7	3.7	6.6	9.3	6.5	6.5	3.7	2.8	5.0
K	10.8	15.6	6.8*	30.1	22.1	14.8	4.2	11.2	30.6	38.0	16.8	33.1	6.4	18.0
Ca	3.1	6.2	5.3	7.4	4.0	6.3	3.0	5.8	4.0	5.3	4.3	7.0	3.8	9.7
Fe	4.6	17.9	6.2	10.9	3.0	2.3	3.2	4.6	2.4	3.0	5.5	7.0	4.4	14.3

*Indicates significance at the 0.05 level between s and u as tested with Bonferroni's method of the multiple *t* test.

Bonferroni method is conservative in identifying significant differences.

Fig. 2 is a representative EDS spectrum for stratified *P. strobus* seeds. The horizontal axis represents the x-ray energy (KeV) characteristic of elements. In general, higher energies are indicative of elements of higher atomic number. The vertical axis registers the number of x-ray counts emitted from each element. Note the eight peaks that correspond with the eight elements listed, respectively. A peripheral computer analyses the energies, the number of counts, and their respective backgrounds and then calculates the percentage of each element in relation to the total elements detected.

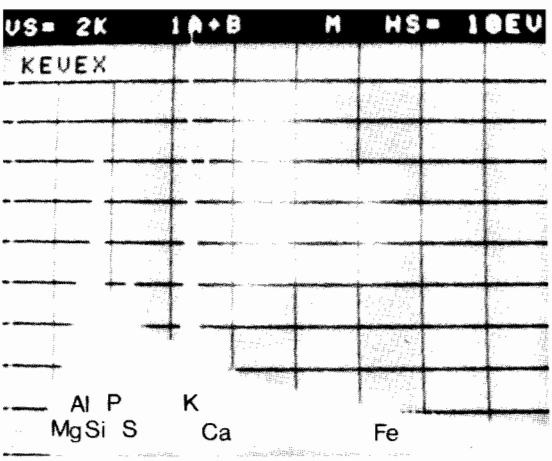

Fig. 2 Representative EDS data collection for stratified seed of *Pinus strobus*. Elemental peaks are labelled on the horizontal axis. The vertical axis registers the number of x-ray counts.

The use of the SEM to provide charged particle excitation of the sample and an energy dispersive spectrometer to accumulate x-ray counts of individual elements provides a unique synthesis of analytical capabilities. SEM provides high-resolution morphological data, and EDS identifies elements present at the surface when it is excited by the scan raster. EDS provides a simultaneous display of all elements present having atomic numbers of 11 (Na) and higher, within minutes. The speed with which analyses can be conducted makes large sample sizes feasible. Probing of small select areas is possible, and differentiation of relatively difficult elements (e.g., K) presents no problem.

Some *Pinus* spp. seeds can be differentiated using seed coat surface elements. Semiquantitative elemental composition can be determined for a signature using state-of-the-art instruments that now allow detection down to carbon (atomic number=6). Distinct EDS advantages allow probing of select, small areas and following of mobilisation of elements during stratification and germination.

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Seed Testing

Germination Pretreatments for Selected Acacia Species from the Pilbara Region of Western Australia

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Abstract

Seed coats of most acacia species are hard and impermeable to water, enabling seed to retain viability under adverse conditions. Some form of pretreatment is required to overcome this physical dormancy and promote rapid and uniform germination. The effect of five treatments on the seed of 13 species recently collected from wild populations in the arid tropical Pilbara region of northwestern Australia was investigated. Methods for breaking seed coat dormancy have not been studied previously for these species.

THE genus *Acacia* includes about 1200 species of trees and shrubs with a natural distribution in Australia, Asia, Africa and the Americas. Over 800 of these species are endemic to Australia. Species from the higher rainfall zones of Australia, in particular those which originate from both the moist temperate regions of the south and the tropical humid zone of the northeast, have been grown successfully in other countries (Turnbull 1986). Such species include *A. mearnsii*, *A. saligna* and *A. mangium*. Interest in the potential of tropical dry-zone species is now increasing, with species like *A. holosericea* and *A. aneura* receiving widespread attention.

The seed of most acacia species is characterised by a 'hard', water-impermeable coat which allows it to remain dormant unless some form of treatment is applied. Potential seed treatments include the use of boiling or hot water, acid, organic solvents, dry heat, microwave energy, impaction, percussion and manual or mechanical scarification (Doran et al. 1983).

Acacia seed varies greatly in size, shape, colour and level of seed coat dormancy, both within and between species. The interaction between pretreatments and degree of hardseededness also varies greatly between species, between seedlots of the same species and between seeds of the same seedlot. The proportion of hard seed in a sample depends on the environmental conditions during the growth of the plant, the degree of maturation of the seeds when collected and the length of the storage period. It is not surprising that workers have found it difficult to prescribe an 'optimum' treatment (or range of treatments) that is highly effective in stimulating germination in most acacia seed (Doran and Gunn 1987).

In October 1988 a seed collecting team from the Australian Tree Seed Centre (ATSC), part of the CSIRO Division of Forestry and Forest Products, undertook seed collections to extend the range of potentially important seedlots from the hot dry Pilbara region of Western Australia (Fig. 1), a latitudinal range of 20° 00′–23° 15′S. Much of the area receives less than 500 mm of rainfall annually, falling mainly between January and March. Temperatures range from a mean maximum of 33°C to a mean minimum of 20°C with some areas of high elevation receiving frost. Soils from massive deposits of Lower Proterozoic sediments and volcanics overlying archean granite and volcanics (Maslin 1982) are infertile. About 120 *Acacia* species dominate the woody vegetation of this zone (Thomson 1989). Of the forty species collected, thirteen were selected for studies of germination since they were considered to have the greatest potential for multipurpose plantings.

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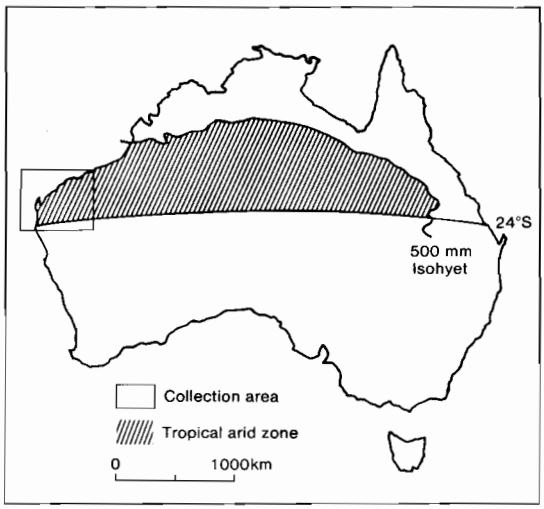

Fig. 1. Seed collection area of the Pilbara Region.

Materials and Methods

Seed collections were made during September and October 1988 when crops were fully mature. Prior to storage the seed was cleaned by hand, thereby largely avoiding inadvertent seed coat damage commonly caused in mechanical threshing. Seed samples from each tree of a given seedlot used in the experiment were bulked. Details of the species tested are given in Table 1. Sixteen seedlots (1 species × 3 seedlots, 1 species × 2 seedlots and 11 species × 1 seedlot) were evaluated between May and June 1989.

Table 2. Description of the presowing treatments for acacia seedlots.

Treatment code	Description name	Treatment
1	Control	No pretreatment. Seed is soaked in water for 24 h at room temperature before sowing.
2	Manual nicking	Using nail clippers/secateurs, a small piece about 1 mm^2 of the seed coat is removed at the distal (cotyledon) end of each seed. Seed is then soaked in water for 24 h at room temperature before sowing.
3	Boiling water, pour and soak	Seed is placed in glass beakers and 10 times the volume of boiling water added. Seed is then left to soak in this water for 24 h at room temperature before sowing.
4	Boiling water, immersion for 1 minute	Seed is contained in perforated metal baskets and immersed in a container of boiling water for 1 minute. Seed is then removed and placed in water at room temperature and allowed to soak for 24 h at room temperature before sowing.
5	Boiling water, immersion for 5 minutes	As for 4, with 5 minutes boiling water.

Table 1. Details of acacia seedlots used in germination trials and most successful presowing treatments.

Species	Seedlot Number (DFFP)	Lat. (S)	Long. (E)	Alt. (*m*)	No. of parent trees	Recommended pretreatments #
A. trachycarpa	16766	21°40′	117°21′	340	15	4
A. trachycarpa	16774	22°24′	118°47′	460	50	2/4/5
A. trachycarpa	16811	20°40′	118°21′	35	40	5
A. citrinoviridis	16817	22°36′	117°37′	620	23	2/4
A. citrinoviridis	16785	23°15′	119°33′	700	25	2/4
A. xiphophylla	16812	20°55′	117°25′	40	7	1/2/3
A. glaucocaesia	16809	20°19′	119°25′	30	15	2/3
A. coriace var. *pendula*	16791	21°00′	118°00′	300	11	1/*
A. atkinsiana	16763	21°36′	117°12′	320	20	2/3/4
A. hamersleyensis	16771	22°45′	118°24′	790	40	2/4
A. aphanoclada MS	16793	21°56′	120°06′	400	60	3/4
A. distans	16772	22°15′	118°42′	407	25	2/3
A. wanyu	16780	23°08′	119°56′	475	30	2/4
A. tenuissima	16773	22°18′	118°41′	420	15	2/3/4/5
A. effusa	16815	22°34′	118°06′	750	15	3/4
A. synchronicia MS	16822	22°25′	115°45′	135	10	2/3

*Recommended pretreatments — acid (H_2SO_4) for 30 minutes; immersion in hot water at 90°C for 1 minute.
#Codes given in Table 2.

For each treatment, 100 apparently healthy seeds (25 seeds × 4 replicates) were taken at random from the bulked seedlot. The seeds were subjected to the presowing treatments described in Table 2. The only exception was *A. effusa* for which there was only sufficient seed for 2 replicates per treatment. After treatment, seeds were soaked in water at room temperature for 24 hours. Each replicate of 25 seeds was then placed in a 9 cm glass petri dish containing vermiculite (7g) moistened with 30 ml distilled water. Dishes were then placed in a germination cabinet set at a constant 30°C with light for 12 hours per day. This temperature is standard for germination for most tropical Australian acacias under laboratory conditions.

Seeds were considered to have germinated once the radicle length was at least the same length as the seed. Germination counts were made at regular intervals for up to 18 days. Germinants were removed from the dishes, extra distilled water added where necessary and seed affected by fungi were treated with a solution of Captan (1.0 g/litre). Fungal attack was so bad in lots where the pretreatment was too severe that a 'squash' test count of viable but ungerminated seeds at the end of the test period was not feasible. The calculation of cumulative germination percentages by seedlot, treatment and time was based on actual germination over total seed sown.

Results and Discussion

Bar graphs for all seedlots showing the mean cumulative germination versus treatment at the conclusion of the 18 day test period are given in

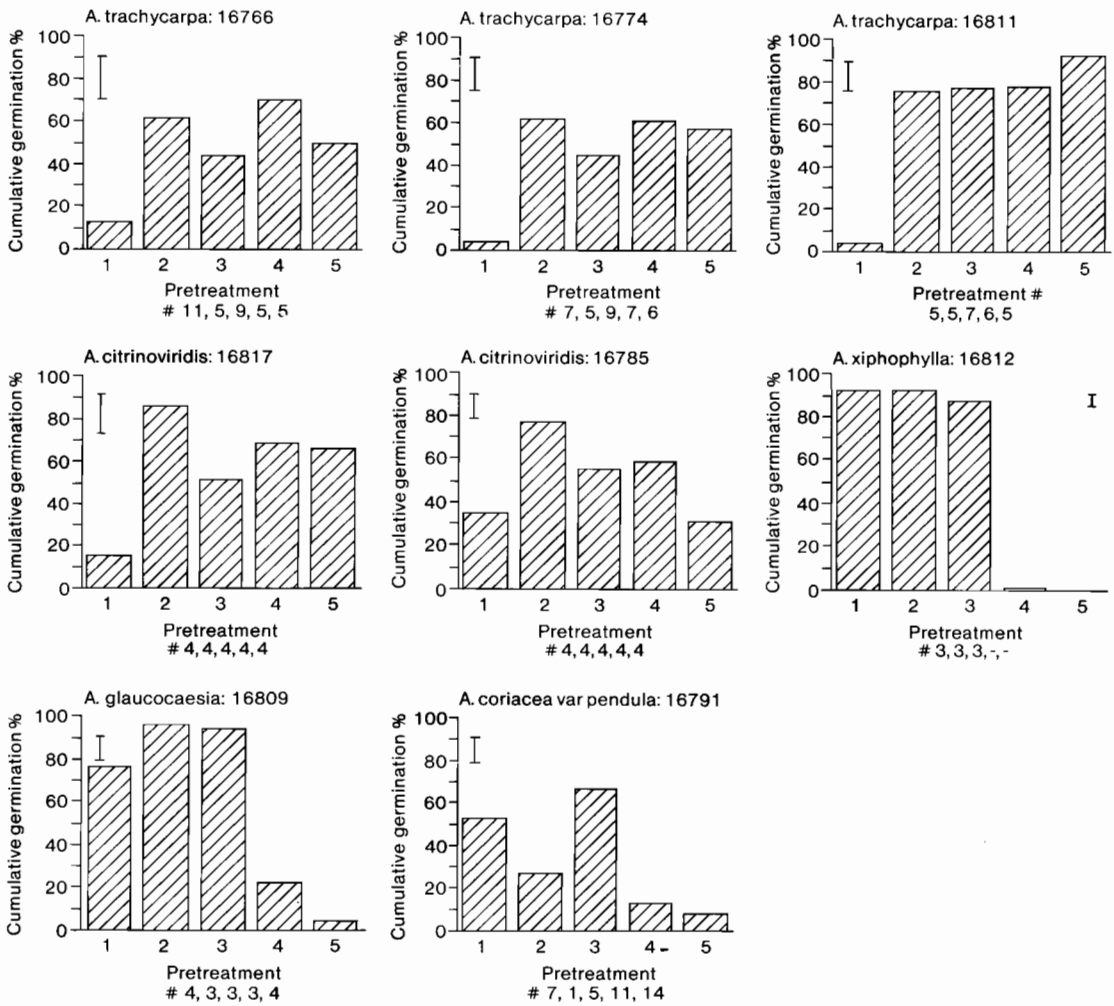

Fig. 2. Mean cumulative germination at 18 days presowing treatment for 16 acacia seedlots.
Days to reach 50% germination.
I Lease Significant Difference (5%) for each mean.

Fig. 2 cont.

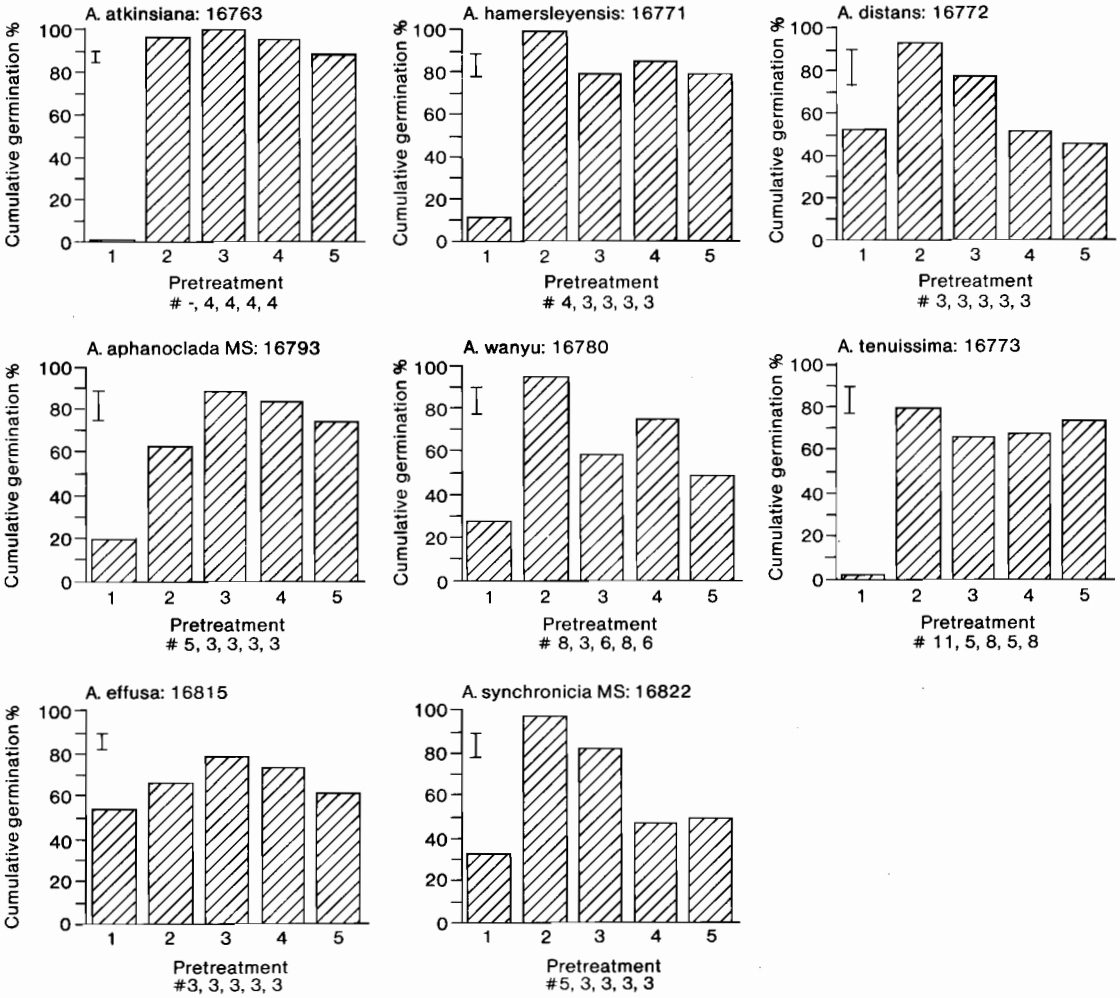

Fig. 2. In addition 'rate of germination' is expressed by the number of days to reach 50% germination. Recommended treatments for each seedlot are shown in the last column of Table 1.

1. Control

Seed subjected to no pretreatment gave poor results. Six species gave cumulative germination results in the range of 1–20%. A further two species produced less than 50% germination whilst *A. glaucocaesia* had 75%. The exception was *A. xiphophylla* for which no pretreatment gave best results with 93% germination in 4 days. This species is one of a number of Australian acacias known to have 'soft' seed coats where harsh pretreatments may be unnecessary or lethal.

2. Manual Nicking

Overall, this treatment yielded the highest germination, with 90% within the first 6 days for 10 species. Conversely *A. aphanoclada* and *A. coriacea* var. *pendula* gave distinctly poorer results; mould may have been a contributing factor. Manual nicking or similar techniques are recommended for pretreating acacia seed before germination tests (ISTA 1985) and when sowing small and valuable research seedlots. The disadvantages are the slowness of application and the care required not to cut away too much of the seed, thus damaging the cotyledons and embryo.

3. Boiling Water, Pour and Soak

This method gave best results in *A. atkinsiana, A. aphanoclada, A. coriacea* var. *pendula* and *A. effusa*, though all species except *A. trachycarpa* and *A. citrinoviridis* gave good results. Rates for 50% germination of the above species were between 3 and 5 days with most germination complete within 10 days.

4. Boiling Water, Immersion for 1 Minute

This method gave similar overall results to treatment 3. *A. trachycarpa* seedlots 16766 and 16774, *A. citrinoviridis* 16817 and *A. wanyu* responded best to this pretreatment while *A. xiphophylla, A. distans* and *A. coriacea* var. *pendula* showed a marked drop in germination. For the first 4 seedlots, 50% germination was reached between 4 and 8 days. Total germination was achieved within 11 days for *A. trachycarpa* and *A. citrinoviridis* whilst *A. wanyu* extended the full 18 days.

5. Boiling Water, Immersion for 5 Minutes

This treatment gave poor results indicating that for this batch of species it was excessively severe. Only *A. trachycarpa* seedlot 16811 responded favourably to this method, with a germination of 93% in 13 days.

In the case of *A. coriacea* var. *pendula* the above 5 treatments did not give satisfactory results. Two additional treatments were therefore applied to this seedlot with favourable results. Undiluted sulphuric acid (95%, 36N) at room temperature for a period of 30 minutes gave 92% cumulative germination within 13 days and 4 days to reach 50%. Seed was also immersed in hot water at 90°C for 1 minute, giving 86% germination after 18 days with 10 days to reach 50%.

Attention should also be given to age of seed. Whilst 6 months is usually considered sufficient time for a hard seed coat to develop, in this experiment seed was tested 8 months after collection. It is possible, however, that some seeds had not fully developed their 'hard' coats, rendering them more susceptible to the harsher pretreatments. It will be of interest to retest these seedlots after further storage.

Conclusions

The interaction between species and seed treatment must be carefully assessed before undertaking germination programs in the nursery, since large differences in germination response may exist. Moffett (1952) reported that 'the percentage germination following nicking may be taken as a close approximation to the potential germination of a seed sample and, as such, forms a useful figure for comparison when assessing the effect of other treatments'. This hypothesis holds true for most species in this study as all but two gave as high or higher germination percentages following nicking than the next best treatment. However, this method is arduous and difficult especially when dealing with small seeds. Mechanical scarification is an alternative but often gives erratic results.

Water treatments are therefore likely to remain the most widely used methods for breaking seed-coat dormancy in Australian acacias. Treatment 4, boiling water for 1 minute, has been adopted by the ATSC as the general prescription for treating 'hard'-coated acacias. In the case of this new suite of species, such a prescription is harmful to seed of *A. glaucocaesia, A. coriacea* vac. *pendula, A. distans* and *A. synchronicia* MS plus the 'soft'-seeded species *A. xiphophylla*. This highlights the problem of defining a standard water treatment effective for most species.

Acknowledgments

I wish to thank L. A. J. Thomson for collecting the seed, providing useful information on species and for comments on the paper, J. Larmour for assisting with the germination tests and A. C. Matheson for providing the analysis of variance.

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Seed Technology Problems of Sandalwood in Indonesia

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Abstract

The sandalwood (*Santalum album* Linn.) is a native species of Indonesia which has prospects for development and high economic value. All parts of the tree, such as its root, stem and leaves, are useful. The development of this species still faces many problems, such as seed sources, techniques of seed production, testing, storage and sowing. Some problems in seed production, testing and storage techniques have been overcome. Nevertheless further experiments are still needed.

SANDALWOOD (*Santalum album* Linn. with its local name Cendana) is a member of the family Santalaceae. This species grows in a wide range of distribution. In Indonesia it grows naturally from Java to East Nusa Tenggara.

Throughout its wide geographic distribution it often shows significant variation in its growth, and in its resistance to pests and diseases.

This species has been used for a long time, especially for producing sandalwood oil (oleum santali), and for timber which can be used for expensive carving. The oil of sandalwood is produced through distillation from almost any part of the tree. Heartwood produces oil with a yield of 4.5–6.5%; sapwood produces ca 1%, while the root produces ca 10%. The oil of sandalwood contains ca 90–94% santalol. The higher the content of santalol, the more expensive the price of the oil (Anon. 1987).

Sandalwood is nominated as one of the priority species in the establishment of industrial plantation forests in Indonesia. The execution of this program faces many problems, such as limited seed sources and seed handling techniques. Seed is available at this time only from scattered natural stands. Seed sources having average density of 10 trees/ha are hard to find. Generally there are around 3 trees/ha with average diameter less than 10 cm (Suwarno and Husaeni 1987).

Seed handling techniques for sandalwood, such as the estimation of the optimum time for collecting seed, the method of seed extraction, pretreatment, seed testing and the way to store seed safely, have not been extensively studied.

Seed Production

Sandalwood seed production faces many problems. Seed sources are limited and the condition of the stands is unacceptable, but the main problems are how to determine fruit maturity and the criteria for measuring physiological maturity.

The criterion for degree of maturity usually used in the field is the fruit colour. The fleshy fruit of sandalwood is physiologically mature when red to black in colour.

Seed extraction is done manually. This extraction method involves putting fruits into a container and rubbing them by hand. The advantage of this method is that the physical damage to the seed is minimised, but it is inefficient and time-consuming for a large quantity of seeds.

The size and weight of sandalwood seeds is very homogeneous. The weight of 1000 seeds is about 147–167 grams (Anon. 1988), around 6000–6800 seeds/kg.

From the Anova of the value of 1000 seeds (as can be seen in Table 1), which used replications of

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Table 1. Anova of the weight of 1000 sandalwood seeds by using different replications.

Source of variance	Degrees of freedom	Sum square	Mean square	F Calc.	F Tabl. 0.05
Treatments	5	686.18	132.24	1.95	3.11
Error	12	846.08	70.57		

Source: Anon., 1988a. Trial Test Report No. 28, FTSTC.

100 seeds each, it is shown that the weight of sandalwood seed is very homogeneous. Seed variation between replications is very small, and samples do not show any significant differences from one another.

Comparing three different seed sizes — large (weight of 1000 seeds ca 214.98 grams), medium (weight of 1000 seeds ca 140.13 grams) and small (weight of 1000 seeds ca 120.67 grams) — indicated that different seed sizes have similar capabilities in germination (Table 2).

Table 2. Anova of the influence of seed sizes to seed germination capacity.

Source of variance	Degrees of freedom	Sum square	Mean square	F Calc.	F Tabl. 0.05
Treatments	2	4.34	2.17	1.12	5.14
Error	6	11.66	1.99		

Source: Anon., 1988b. Trial Test Report No. 42, FTSTC.

Seed Testing

Sandalwood seed is very difficult to germinate, and its initial germination percentage is often very low. However, a report by Masano (1986) gives a germination capacity of 70% (picked mature fruits) and 85% (seed in bird droppings). Information from India is that open pollinated seed has a germination of 45% (Richmond 1987). Poor germination is experienced because: (1) the seed has a hard seed coat or has seed coat dormancy, and (2) a certain light intensity seems to be necessary for germination of the seed.

A trial test for breaking seed coat dormancy has been carried out using gibberellic acid (GA_3). In this trial test, different concentrations of GA_3 have been tried and the seed planted in the greenhouse and laboratory. The medium used for testing seed in the greenhouse was a mixture of soil and sand (1:1), while the medium used for testing seed in the laboratory was activated carbon cotton. The results of this trial test (Table 3) show that a 0.10% solution of gibberellic acid can produce a ninefold increase in germination percentage in greenhouse trials, while a 0.05% solution produced a sixfold increase in germination percentage in laboratory trials, indicating the usefulness of gibberellic acid in breaking seed coat dormancy.

Table 3. Germination (%) of sandalwood seed by using different concentrations of GA_3.

Place of testing	Concentration of GA_3 (%) Control	0.025	0.05	0.10
Greenhouse	5	34	26	46
Laboratory	11	49	60	15

Source: Anon., 1989. FTSTC, Unpublished.

To prove that the sandalwood seed needs light for its germination, Suginingsih (1987) placed the germination box under different light intensities (full, half open and shady). She also tried the same experiment without pretreatment and after placing seeds in hot water at 60°C, allowing them to cool then holding in cold water for 24 hours before sowing. The experiment indicated that the seeds of sandalwood should be soaked in hot water and need full light to enhance germination.

Seed Storage

Sandalwood seeds which have just been extracted always have high moisture content of ± 27%. If the seed is to be stored, its moisture content should be decreased.

The result of the test indicated that the methods of seed drying to reduce seed moisture content using sunlight for 1–3 days or seed drier (40°C, RH 50%) for 8–24 hours did not significantly influence seed quality as measured by germination percentage (Table 4). It can be concluded that either method of drying can be used for decreasing the moisture content of sandalwood seed.

Sandalwood seed can be stored safely with low moisture content. An experiment carried out using sandalwood seed with different levels of moisture content (i.e. 3–4, 5–6, 7–8, 9–10%) packed into plastic bags in a sealed tin container after adding some wood ash as a storage medium and the fungicide Dithane M45. The wood ash added was half the weight of the seeds, while the fungicide was 0.2% of the weight of wood ash. The plastic bags were placed either in dry cold storage (DCS, ca 4°C; RH 40–50%) in an air conditioned room (CR, 18–22°C, RH 60–70%) or in ambient conditions (25–28°C, RH 70–90%) for 2, 4, 6 to 18 months.

The initial germination percentage was about 66–69%. The results of the experiment indicated

Table 4. The LSD Test for the value of moisture content (%) and germination (%) before and after drying activity.

Drying Method	Moisture content (%)[1)]	Germination percentage (%)
Without Drying	27.58[a]	23[g]
Sun drying for 1 day	5.00 [b]	37[g]
Sun drying for 2 days	3.98 [c]	27[g]
Sun drying for 3 days	3.30 [d]	20[g]
Seed drier (40°C), 8 hours	2.86 [f]	30[g]
Seed drier (40°C), 16 hours	2.13 [f]	31[g]
Seed drier (40°C), 24 hours	1.97 [f]	22[g]

[1)] = moisture content determination based on the wet weight, and it is done by putting the entire seeds into oven of 105°C for 24 hours.
Source: Anon., 1988c. Trial Test Report No. 54, FTSTC.

that sandalwood seed with low moisture content (3–10%) can be stored for 16 months in DCS (GP = 66%) and CR (GP = 67%), while in ambient conditions the seed was only able to maintain its viability for 4 months (GP = 65%). After these periods, the viability of the seeds decreased sharply (Table 5).

Conclusions

From these experiments it can be concluded:

1. Sandalwood seed is relatively uniform in size and the differences in seed size do not influence germination capacity.
2. Seed coat dormancy can be broken by treat-

Table 5. Newman Keuls Test for the average value of germination percentage of sandalwood seeds stored in different storage conditions.

Storage conditions	Storage period (months)	Germination (%)
Dry cold storage (DCS)	2	52.50 DEFG
	4	69.33 G
	6	40.84 CDE
	8	46.83 CDEFG
	10	61.33 EFG
	12	40.83 CDE
	14	54.83 DEFG
	16	66.50 DEFG
	18	4.50 A
Conditioned room (CR)	2	61.50 EFG
	4	63.17 EFG
	6	52.67 EFG
	8	71.00 G
	10	65.00 EFG
	12	45.34 CDEF
	14	42.00 CDEF
	16	67.17 FG
	18	5.84 A
Ambient	2	58.50 CDEFG
	4	65.33 EFG
	6	29.50 BCD
	8	26.17 ABC
	10	9.33 A
	12	7.50 A
	14	14.50 AB
	16	12.17 A
	18	1.00 A

Source: Anon., 1988d. Trial Test Report No. 55, FTSTC.

ment with 0.05% GA_3 or by placing the seed in hot water (60°C) which is then allowed to cool for 24 hours.

3. Seed drying to reduce moisture content for storage purposes can be done under sunlight for 1–3 days or by using a seed drier at 40°C for 8–24 hours.
4. Sandalwood seed can be stored safely at low moisture content (3–10%) in storage conditions of low temperature and relative humidity (± 4°C; RH ± 40%) for up to 16 months.

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Methods for Seed Quality Control for *Pinus elliottii* var. *elliottii*

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Abstract

The main object of the present study was to decide on an expedient laboratory method for seed performance evaluation so as to furnish nurserymen with more accurate and meaningful information. Seed lots of *Pinus elliottii* Engelm. var. *elliottii* were collected from two seed stands, known as 'Chacra 3' and 'Represa', located in Bañado Medina Experiment Station, College of Agriculture, Cerro Largo, Uruguay. The four procedures assayed included three different laboratory tests, viz. a) standard germination test; b) aging test and c) tetrazolium test, which were complemented by sanitary analysis. A fourth treatment included d) field (nursery) emergence test.

THE demand for wood as a raw material and wood products has increased substantially during recent years in Uruguay. Such trend has emphasised the need of a reliable supply of quality forest seed. Nurseries and tree planters acquire *Pinus elliottii* Engelm. var. *elliottii* seed from the seed bank at the College of Agriculture in Montevideo. The seed bank markets commercial seed lots only after these have undergone analysis and certification for germination and quality. Nevertheless, current tests usually fail to adequately estimate future seed performance in field (nursery) conditions. The present study aims to assess results obtained using several test procedures and explain why these methods differ in outcome when applied to the same seed lots.

The object of the present study is to identify an adequate correlation between laboratory test results using several procedures—viz. standard germination test, tetrazolium viability test, seed aging test — and field (nursery) emergency, to identify an expedient and accurate test method with increasing periods of prechilling treatments.

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Seed Testing

Standard Germination Test

Standard germination testing yields information concerning seed quality which enables comparison between seed lots. However, germination test results usually do not correlate accurately with field (nursery) emergence, as nursery conditions are often quite different from laboratory conditions. (Congreso Internacional de Ensayos de Semillas 1974).

Viability Test with Tetrazolium

The tetrazolium viability test is a biochemical procedure involving the staining of living cells. The reactant substance is a tetrazolium salt solution, which is absorbed by the seed and penetrates the tissues. Tetrazolium takes the hydrogen freed from reduction processes induced by dehydrogenases in the living cells. The hydrogenation of tetrazolium salt results in a red compound (triphenylformazan) which enables visual identification of living portions of seed (Congreso Internacional de Ensayos de Semillas 1974).

The purposes of this test method are: a) to promptly assess seed viability; b) to assess viability of dormant seed which do not germinate in the standard germination test; c) to assess viability of low performance seed lots after inadequate

storage or poor sowing conditions. (Congreso Internacional de Ensayos de Semillas 1974; Moore 1984).

As main disadvantages of the method, Moore (1984) recognised the need for personnel training, the ardousness of procedure and its failure to show infection and/or dormancy.

Seed Aging Test

The importance of this test lies in its ability to reflect future field (nursery) emergence as it is a vigour test (Szabo and Dolinka 1988). Vigour methods are uncomplicated, expedient, objective, cheap, easily repeatable and closely related to field (nursery) results (Copeland and MacDonald 1985). Seed aging is used to assess seed quality (Barkin 1981) and also reflects seed storage conditions (Szabo and Dolinka 1988).

Seed Health Testing

Seed-borne pathogens influence germination test results. Contaminated seed lots have been identified as main pathogen carriers within great distances (Anselme 1981). An abundant growth of storage fungi and saprophytes indicates low quality in seed lots, due to poor harvest techniques, inadequate storage conditions or aging (Congreso Internacional de Ensayos de Semillas 1974).

Nursery Emergence Test

This test yields information on the real quality of nursery germinating seed (Flinta 1966). The method involves sowing seed lots under currently recommended normal nursery conditions (Popinigis 1975).

Materials and Methods

The seed lots were collected from two seed stands growing at the Bañado Medina Experiment Station, Cerro Largo, Uruguay. Seed lots were collected in February-March 1988 from stands of *Pinus elliottii* var. *elliottii*, known as Chacra 3 and Represa.

Seed lots underwent standard germination, viability, and seed aging tests, complemented by seed health testing monthly. Each working sample was then taken from the refrigerator, where they had been stored at 8–10°C, so that monthly sample received an additional month's prechilling treatment, through the test period from June to October.

Seed health testing, seed aging tests and nursery emergence tests were carried out on 400-seed lots. The same procedure was held for viability tests, but using 200 seed per lot.

Germination substrate used for standard germination and aging tests and for seed health testing was blotter paper moistened with distilled water and contained in sterilised Petri dishes. A Burrows germination cabinet was used for all three methods. The cabinet was set with two cycles, a cool cycle at 25°C without light and a heat cycle at 30°C with light provided by Daylight tubes. The records were kept according to ISTA specifications for germination assessment on days 7 and 28 after sowing. The seed aging test was carried out with 400 seeds from each seed lot, divided into two sets of 200; each set was placed in a flask containing distilled water up to 25% of its capacity (i.e. 25ml). The seed was held, exposed to high humidity at 29°C during 48 hours, inside the germination cabinet by means of plastic sieves. After this period, seeds were sown as for the standard germination test.

Sanitary analyses were carried out on day 12 after sowing in the same way as for the standard germination test. Petri dishes were observed under a Wild stereo-microscope; the pathogens thus observed were then identified under an Olympus microscope.

For the tetrazolium viability test, 200-seed samples were soaked for 24 hours in distilled water after piercing the testa. Each testa was then entirely removed and the seed was soaked in the tetrazolium solution (1%) and held at 36°C for 24 hours in the oven. Afterwards, the seed was observed.

Nursery emergence was assessed by means of a 400-seed sample, sown in 4 rows; emergence records were kept each day after sowing. Seed samples were watered daily until the germination process stopped. Emergence speed was computed monthly for each seed lot as:

$$\text{Emergence speed} = \Sigma \frac{\text{number of emerged plants during the day}}{\text{number of days after sowing}}$$

Results and Conclusions

- The Chacra 3 seedlot results indicate that the best methods for estimating future nursery emergence were viability tests held from June to October and the standard germination test held in August. The most expedient procedure of forecasting nursery emergence was the tetrazolium viability test held in June (see Table 1).
- The Represa seedlot results indicate that the most convenient tests for estimating future nursery emergence were viability tests held in June, July, September and October and standard germination tests held from August to October. Among these, the most expedient procedure for forecasting nursery emergence was the tetrazolium viability test held in June (see Table 1).
- The main effect by month can be ascribed to prechilling time. Prechilling treatment allows

Table 1. Average test values. (A) standard germination test (%); (B) aging test (%); (C) viability test (percentage of viability) and (D) field (nursery) emergence test (percentage of emergence).

	Seed Stand									
	Chacra 3					Represa				
Treat.	Jun.	Jul.	Aug.	Sep.	Oct.	Jun.	Jul.	Aug.	Sep.	Oct.
A	38	29.50	63	52.25	49.75	30.25	41.25	73.25	61.50	65
B	50.50	24.25	—	53.75	53	28	30.75	—	74.75	73.50
C	70.50	74.50	85	73.50	71	69	75	82.50	73	74
D	76.50	49.50	50.25	38.75	56.25	66	50.50	51.50	58.25	60.75

recently collected *Pinus* seed to gradually achieve maturity and break dormancy, thus favouring germination.

- Laboratory test results indicate that the best germination percentage was achieved in August, as a consequence of the optimisation of seed maturity conditions (see Table 1).
- Seed aging test results suggest that the actual effect is not aging, but rather an acceleration of physiological maturing processes, producing a slight increase in germination percentages.
- Percentage germination was observed to decrease with increasing incidence of pathogen infection.
- Nonsignificant differences were observed between emergence speed values for seed stand lots, in favour of the Represa samples (see Table 2). Emergence speed was higher during September and October compared to June, July and August values. On the other hand, percentage of emergents was higher in June (see Table 2). This high emergence capacity may be due to the lengthy period (almost three months) during which seed sat in the nursery without germinating. This had the effect of a prechilling treatment in natural conditions. Prechilling at low temperature and high humidity achieved a breakage of dormancy, a faster maturity and a higher degree of emergence homogeneity. Low temperatures allowed for healthier conditions as they restricted pathogen development.
- Finally, under trial conditions the best time for seed sowing in the nursery appeared to be June–July. Even though this period implies a more intensive cultural practice, a better emergence is achieved. Spring sowing, which showed the fastest rate of emergence is, however, a season where a higher susceptibility to pathogen infection occurs.

Table 2. Speed of emergence. Percentage values for both stands.

Month/stand	Chacra 3	Represa
June	0,75	0,698
July	0,574	0,662
August	0,897	0,999
September	1,425	2,414
October	2,279	2,893

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Testing Chinese Fir Seeds by Seedling Vigour Classification on Vertical Plates

Chen Yousheng and Chen Zhijian*

Abstract

Fifty samples of Chinese fir seeds were tested using seedling vigour classification in a vertical plate germinator. Seedlings produced on vertical plates were classified into five classes, each representing a stage of germination. Equations were given for predicting nursery establishment and plant percentage in two agroclimatic conditions and for detecting genetic vigour of seeds among families by seedling vigour classification tests in a 20-day germination period.

CHINESE fir, *Cunninghamia lanceolata* (Lamb.) Hook, a fast growing tree species with wide natural distribution, high productivity and good quality of wood, is the most important timber tree species in southern China. It makes up 20–25% of annual timber production. Although the ever increasing interest in Chinese fir has led to intensified research on its seeds, only one study of electrical conductivity has related to seed vigour (Fu 1984). Many seed vigour tests have been developed, but the seedling vigour classification test is the only one which can be practised in ordinary seed testing laboratories, due to its ease of scoring and simplicity (Perry 1981; Wang 1973). Modifying Wang's criteria for seedling vigour classifications, this paper studied the seed vigour with a laboratory-made vertical plate germinator to evaluate the nursery performance of Chinese fir.

Materials and Methods

The seeds used in the present study were collected from 12 families separately in Yangkou Forest Station, Fujian Province, in December, 1985. The seeds were artificially aged for various periods at 40°C with relative humidity about 100%, which resulted in 50 samples with germination ranging from 4 to 68%.

Sowings of 4×150 seeds of each of the 50 samples occurred in randomised blocks at the following nurseries: Lishui, Fujian; Xianning, Hubei; Huangpi, Hubei; Jurong, Jiangsu. Lishui is located in the southern part of the subtropical region, while the others are in the north subtropics. Sowing and management followed local practice. On the basis of number of the seeds sown, nursery establishment of each sample was checked before summer when it was believed that few additional seedlings would emerge. Collar diameter and seedling height were measured and plant percentage, based upon the number of seeds sown, was calculated in autumn in Lishui only.

Seed vigour was tested in the vertical plate germinator, $26 \times 21 \times 21$ cm, in which 10 organic glass plates are vertically inserted 12 mm apart. Before inserting the plates, seeds were sown in a line on the plate 6 or 7 cm below its upper edge. The plate with the sown seeds was fully covered with a piece of filter paper. A moderate mist spray applied to the paper cover held the seeds firmly on the plate. Nine plates were sown with 3×100 seeds of each of the 50 samples. A water depth of about 2 cm was maintained in the germinator so that seeds could absorb sufficient water through filter paper to enable them to germinate. Germinators were shelved in a dark room where temperature was kept at constant 25°C according to the

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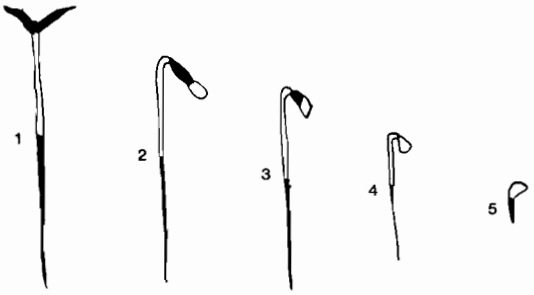

Fig. 1. Chinese fir seedling vigour classes on vertical plate: 1) fully developed seedling with seedcoat completely shed; 2) well developed seedling with seedcoat almost completely shed; 3) emerged radicle and raised hypocotyl with seedcoat slightly shed; 4) emerged radicle and raised hypocotyl with cotyledon not yet visible; 5) emerged radicle longer than half the length of the seed.

Rules for Testing Tree Seed (China Standardization Bureau 1982).

Seedlings produced on the plates were scored daily according to the criteria shown in Fig. 1. Abnormal seedlings were considered as ungerminated, except polyembryo seeds of which one usually developed into a normal seedling. Seedlings with radicle slightly shrunk or curled, or with either radicle or hypocotyl growing slowly were rated into a lower class.

Results

1. Dependability of Nursery Performance upon Seed Vigour

Nursery performance of a seed sample depends not only upon its vigour but also upon the environmental conditions and nursery practice. Factor analysis was used in the present study to detect the degree of seed vigour in determining the nursery performance which was termed 'dependability of nursery performance upon seed vigour'. The larger the significance (F) and variance contribution (%), the more the seed vigour contributed to nursery performance.

The dependability of nursery performance upon seed vigour of the 50 samples in the four nurseries is shown in Table 1 in sequence from large to small. It was shown that in Lishui more than 60% of the difference in nursery performance among seed samples came from the differences in seed vigour. On the contrary, the effect of seed vigour on nursery performance was very small (<1%) in Huangpi nursery because of the less favourable environment and poor nursery practice.

2. Termination of the Seed Vigour Classification Test

The trend of the cumulative number of seedlings (CANS) developed from lower vigour classes into higher ones in each sample was examined to determine the end of the test period. The CANS in four of the 50 samples are illustrated in Fig. 2. as an example. In the medium quality samples represented by No. 42 and 45, the CANS had no significant change up to day 18 or 19 after incubation, while in high quality samples represented

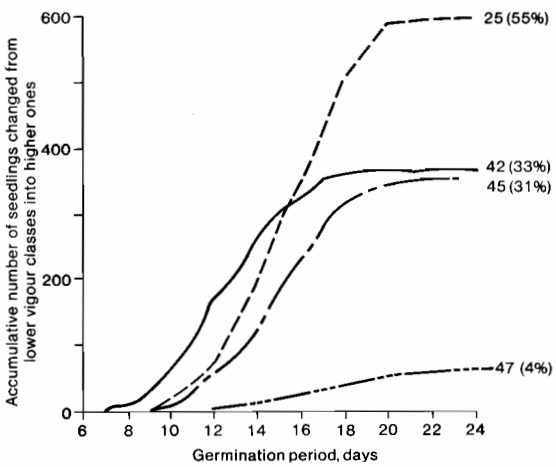

Fig. 2. Accumulative number of seedlings in four samples changed from lower vigour classes into higher ones during test period on vertical plate. Normal germination percentages are shown in parentheses.

Table 1. Dependability of nursery performance upon seed vigour in Chinese fir.

	NE-L#	Plant % (L)	Seedling diameter (L)	Seedling height (L)	NE-J#	NE-X#	NE-H#
Significance, F	7.158**	4.083**	2.946**	1.088	2.067**	1.709**	1.032
Variation contribution (%)	60.62	43.52	32.73	1.24	21.06	15.00	0.79

#NE, nursery establishment. L, J, X and H represent each of the four nurseries namely Lishui, Jurong, Xianning and Huangpi, respectively.
**Significant at 0.01 level.

Table 2. Significance (F) tested by multiple regression analysis between nursery performance and seed vigour within different germination period.

Germination period, days	NE-L#	Plant % (L)	Seedling diameter (L)	Seedling height (L)	NE-J#	NE-X#	NE-H#
21	29.667**	18.598**	7.460**	2.000*	9.168**	11.033**	1.281
20	34.760**	18.827**	8.133**	0.957	7.192**	1.684*	0.613
19	25.838**	14.786**	7.661**	0.898	7.271**	14.820**	1.102
18	25.498**	17.492**	7.882**	0.896	8.162**	12.829**	0.159
17	22.624**	4.950**	8.127**	1.074	9.238**	11.872**	0.213
16	22.014**	16.065**	8.243**	2.219*	14.077**	1.806*	0.628
15	22.356**	16.280**	8.321**	0.730	11.634**	11.614**	0.399
14	17.964**	9.959**	8.303**	1.595	8.038**	10.970**	0.464
13	23.882**	16.786**	8.153**	0.904	10.949**	12.253**	0.568
12	21.477**	18.669**	8.107**	1.008	8.592**	6.630**	1.266
11	22.748**	16.567**	8.101**	1.038	7.813**	9.670**	0.652
10	16.355**	13.673**	7.476**	0.939	6.477**	8.439**	1.091

#cf. the footnote under Table 1.
**Significant at 0.01 level. *Significant at 0.05 level.

by No. 25 and low quality samples represented by No. 47 the curves of the CANS appeared to be smooth and steady beyond day 20. It seemed to be reasonable to terminate the seed vigour classification test on day 20 for Chinese fir.

Tests of significance were done using multiple regression analysis between the nursery performance of the 50 samples and their seed vigour expressed on vertical plates from 10 to 21 days (Table 2). It can be seen from Table 2 that only the F values on day 20 are in the same sequence as those shown in Table 1. This further proves that the number of seedlings accumulated in vigour classes up to 20 days after the beginning of incubation on vertical plates is an index reflecting nursery performance of Chinese fir seed. In other words, the vigour of a seed for germination and subsequent seedling growth as expressed in vigour classes I-V within a 20-day test period, is a sensitive indication for predicting nursery performance. It therefore seems reasonable to terminate the seed vigour classification test in this instance after a 20-day period.

3. Prediction of Nursery Performance

In the vertical-plate germination test, the number of germinants in a seed sample reaching a certain stage of seedling development in the 20-day period is a random and independent variable that can be used to establish multiple regression equations with nursery performance. An equation was established for predicting nursery establishment in Lishui Nursery, where the climatic conditions are favourable for Chinese fir and people are skilful in nursery practice:

$$Y = 100 \times \sin\ (0.007297 + 0.561327 \text{ arc sin } X_1 + 0.483124 \text{ arc sin } X_2 - 3.041500 \text{ arc sin } X_3 + 1.846653 \text{ arc sin } X_4 + 1.434584 \text{ arc sin } X_5) \quad \text{(Equation 1)}$$

where Y—nursery establishment (%), and X_1, X_2, X_3, X_4 and X_5—the percentage of seedlings in classes I, II, III, IV and V, respectively.

For Lishui Nursery, the equation for predicting plant percentage is as follows:

$$Y = 100 \times \sin\ (0.001065 + 0.323214 \text{ arc sin } X_1 + 0.321916 \text{ arc sin } X_2 - 2.264455 \text{ arc sin } X_3 + 1.280330 \text{ arc sin } X_4 + 0.825670 \text{ arc sin } X_5) \quad \text{(Equation 2)}$$

where Y—plant percentage, and X_1, X_2, X_3, X_4 and X_5—as in equation (1).

For the other three nurseries where the climatic conditions are less favourable for the species and people are less skilled in nursery practice, the equation fitted for predicting nursery establishment is as follows:

$$Y = 100 \times \sin\ (0.008208 + 0.064016 \text{ arc sin } X_1 + 0.181445 \text{ arc sin } X_2 - 0.627524 \text{ arc sin } X_3 - 0.114923 \text{ arc sin } x_4 + 0.110902 \text{ arc sin } X_5) \quad \text{(Equation 3)}$$

The partial correlation coefficients of equations (1),(2) and (3) are 0.89, 0.83 and 0.65 respectively, proving the accuracy of the equations. Taking 6 samples, Table 3 shows that the predicted values in the three equations agree well with the real values in nurseries. The greatest difference between them is only 3.1%.

4. Detecting the Difference of Genetic Vigour

There are two aspects of vigour — genetic and physiological (Bruce and Eric 1972). The genetic vigour determines the potential of physiological vigour and its decreasing speed in stress conditions.

Table 3. Comparison of values predicted by equations (Y_p) with those observed in nurseries (Y_o).

Seed sample No.	Percentage of seedlings in vigour class (%)					Equation (1)		Equation (2)		Equation (3)	
	X_1	X_2	X_3	X_4	X_5	Y_p	Y_o	Y_p	Y_o	Y_p	Y_o
1	53.3	8.0	2.0	0.7	0.0	30.2	28.7	17.0	15.0	3.7	3.1
2	54.0	11.3	0.3	1.7	0.0	38.7	38.2	23.4	25.3	5.3	3.8
3	18.7	2.0	2.0	1.7	2.3	11.9	11.8	6.3	7.0	0.4	0.2
4	53.0	7.0	3.0	1.7	1.0	29.8	27.2	16.4	13.3	2.9	1.3
7	33.3	2.7	0.3	0.0	0.0	19.3	20.5	11.1	10.9	2.5	1.3
8	27.7	3.3	1.0	0.7	0.0	15.6	13.5	8.8	7.8	1.7	2.0

It follows that after the seed samples have been artificially aged, the difference of genetic vigour among families can be detected by a seedling vigour classification test on vertical plates.

For example, with aging treatments in various degrees under the same conditions (40°C with relative humidity about 100%), obvious changes can be observed in the seed vigour among the four families expressed by equation (1) (Fig.3). The seed vigour of family 8421 had a decreasing rate almost the same as that of family 33. The seed vigour of families 47 and 33, though almost in the same level before treatment, has shown appreciable difference after the aging treatment. The greatest rate of decrease was seen in family 29 whose vigour before treatment was ranked second among four, but appears to be falling to the lowest level.

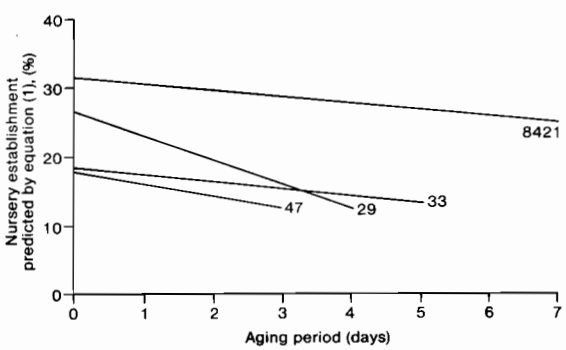

Fig. 3. Difference in genetic vigour among four families of Chinese fir seed.

Discussion

1. Criteria for Seedling Vigour Classification

An objective criterion is necessary in seedling vigour evaluation. In this study the stages of seedling development are believed to be a reasonable expression of seed vigour, as compared with those suggested by Perry (1981) or by the Seed Vigour Test Committee of AOSA (1983). Although easy to carry out on the vertical plate, the seedling growth rate test (Perry 1981; AOSA 1983) was not conducted in the present study. It was noticed that to a certain extent growth rate of the radicle or hypocotyl seemed to be a genetically controlled trait.

2. Evaluation of the Tests

Compared with the linear regression analysis made by Wang (1973), especially with those made by Yu (1958) and Zhang (1985) who assumed that there existed a linear relationship between germination percentage and nursery emergence, the multiple regression analysis and the double continuous curve in 6-dimensional space used here provides a better way to describe vigour in a seed sample. As a result, the predicted values given by equations (1), (2) and (3) fit very well with the real values observed in nurseries (see Table 3).

The routine germination test fails in some important ways as a measure of the plant-producing ability of seed due to philosophical and methodological inadequacies (AOSA 1983). It is believed that the seedling vigour classification test on vertical plates can meet the needs of seed users whose main concern is nursery establishment and plant percentage from a seedlot.

The vertical-plate germination test is simple and practical, with less modification in the routine procedures and no additional expensive laboratory equipment.

The coefficients of X_3 in equations (1) and (2) and that of X_3 and X_4 in equation (3) are negative. This implies that the larger the percentage of seedlings in class III or IV, the lower is the seed vigour. In other words, the poorer will be the nursery performance of the seed sample (cf. Table 3).

In combination with artificial aging treatment, several vertical-plate germination tests in succession are capable of evaluating the difference of genetic vigour among seed samples (cf. Fig. 3). It can be speculated that the values from the tests revealed the resistance to stress, especially the storability of the seed. Among the four families shown in Fig. 3, No. 29 seemed to have the poorest

stability in storage. Therefore it appears that the seedling vigour classification test on vertical-plate has a potential value in monitoring control in seed storage.

More work is needed to prove the seedling classification test on vertical plates as well as the seedling vigour evaluation criteria for Chinese fir and other tree species in more nurseries under various conditions.

Acknowledgments

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Accelerated Aging: a Potential Vigour Test for Multipurpose Tree Seeds

W.W. Elam and C.A. Blanche*

Abstract

This paper presents a summary of our four-year efforts in evaluating the applicability and utility of the accelerated aging technique (AA) as a means of assessing the relative vigour of tree seeds. The optimum accelerated aging conditions were determined for each species investigated. Physiological changes accompanying the accelerated aging process were monitored in an effort to gain a better understanding of this process. Advantages of this technique and the problems associated with it are presented.

HIGH-VIGOUR seeds store better than low-vigour ones. Therefore, determining the degree of seed lot deterioration is essential in deciding which lots should be utilised first and which ones may be stored for an extended period of time. Because a standard germination test may not readily reveal the vigour of seed lots, the use of vigour tests, one of which is the accelerated aging technique, is recommended. The accelerated aging (AA) technique is a stress test (Association of Official Seed Analysts 1983) whose basic premise is that high-vigour seed lots will show only a slight decline in germination while low-vigour lots will decline markedly after exposure to AA.

Since AA functions by exposing seeds to the two most important environmental variables governing seed deterioration — high temperature (40–45°C) and high relative humidity (>90%) — results of this test more closely reflect seedlot performances under field conditions. Whereas this technique has been extensively investigated with agronomic crop seeds, its applicability with tree seeds has been little studied. Bonner (1974a), however, has used it in creating varying levels of vigour of cherrybark oak (*Quercus pagoda* Raf.) seed lots, while Pitel (1980) reported some biochemical changes during AA of jackpine (*Pinus banksiana* Lamb.) and northern red oak (*Quercus rubra* L.) seeds.

The overall objective of our studies was to determine the applicability of the accelerated aging technique as a means of assessing relative vigour of tree seeds. This paper summarises the results of our four-year efforts to this end. More detailed accounts of these studies have been presented elsewhere (Blanche et al. 1988a, 1988b, 1989; Elam 1988; Matta and Blanche 1989; Marquez-Millano 1987).

Materials and Methods

Seed Source and Collection

Seeds used in these studies were obtained from the Tree Seed Laboratory of the Southern Forest Experiment Station, U.S. Forest Service; the Mississippi Forestry Commission; the International Forest Seed Company; or from local collection. Slash pine (*Pinus elliottii* Engelm.) seeds were from three composite seed lots collected in the southeastern United States (year unknown) with 80, 73, and 89% germination capacities, respectively, in 1985 and two seed lots collected from Florida and Mississippi seed orchards in 1985 with germination capacities of 95% for each lot. Longleaf pine (*Pinus palustris* Mill.) seed lots were obtained from the International Forest Seed

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Company and had been produced in the states of Alabama and Florida. Water oak (*Quercus nigra* L.) seeds were collected on November 24, 1986, from mature trees on the campus of Mississippi State University. Pecan (*Carya illinoensis* (Wangenh.) K. Koch) seeds were those of commercial varieties 'Schley', 'Desirable', and 'Stuart', and two native selections collected in 1987 on the campus of Mississippi State University. Five loblolly pine (*Pinus taeda* L.) seed lots were obtained from the Mississippi Forestry Commission and the Southern Forest Experiment Station of the U.S. Forest Service, all were collected in the southeastern United States.

Accelerated Aging Procedure

All seeds were first surface-sterilised with 2.7% sodium hypochlorite and rinsed thoroughly with sterile distilled water. Accelerated aging was performed initially using different trial temperatures (39, 41, 43, and 45°C) and subsequently all experiments were conducted at 41°C and near 100% RH using a Stults Accelerated Aging Chamber capable of maintaining desired temperatures within ± 0.5°C. Seeds were placed in the aging containers, which are 14 × 14 × 5 cm plastic boxes with tight covers each containing a bronze wire mesh seed holder. Seeds were arranged in a single layer on the wire mesh 1 cm above 100 ml of distilled water. The boxes were then placed in the accelerated aging chamber and were exposed for specified time periods (48, 96, 144, 192, 240, and 288 hours). After treatment applications, seeds were immediately tested for germination unless low-temperature stratification was required for the species. Unaged seeds served as the control. Unless otherwise specified all treatments were replicated four times.

Germination Tests

All germination tests were conducted using Stults germinators and in accordance with the rules for seed testing (Association of Official Seed Analysts 1981) except for pecan and longleaf pine where some modifications were made. Longleaf pine seeds were tested at alternating 20–30°C temperatures (better germination response) in contrast to a constant 20°C temperature as prescribed by the Association of Official Seed Analysts (1981). Pecan seeds were germinated at a constant 34°C (van Staden and others 1976) with alternating dark and light regimes of 16 and 8 hours, respectively. All tests were run for 28 days except for longleaf pine which was run for 21 days. Germination capacity was expressed as percentage germinated of viable seed, while completeness and rate of germination were expressed as germination values (GV) as described by Czabator (1962).

Seed Stratification

Water oak, pecan, and loblolly pine seeds were stratified moist in polyethylene bags at 3°C for 30, 68, and 30 days, respectively.

Moisture Content Determination

Seed moisture content was determined according to Bonner (1974b) for water oak and pecan, and according to Bonner (1979) for the pines.

Leachate Conductivity Measurement

Leachate conductivity of pine seeds was measured using either a multiprobe automatic seed analyser (Model ASAC 1000) or a single probe conductivity meter (YSI Model 35). Seeds were soaked for 24 hours and then were removed prior to reading the leachate conductivity.

Chemical Analyses

Seeds subjected to AA were analysed for reducing and nonreducing sugars, starch, amino N, and total N. Analysis for lipids and individual fatty acids are in progress for pine seeds. A more detailed account of these analyses was presented elsewhere (Blanche et al. 1988a).

Results and Discussion

Responses to Aging Temperature

Initial assessment of temperature for aging using water oak seeds revealed that 39°C did not cause any appreciative aging even for as long as 20 days (480 hours). Using 45°C resulted in the 'cooking' of the acorns as indicated by an odour similar to that of boiled peanuts. With loblolly and slash pine seeds, 39°C did not cause any decline of vigour (germination capacity after AA) for as long as 12 days (288 hours), and temperatures higher than 41°C (43 and 45°C) reduced vigour very drastically (Marquez-Millano 1987) thus limiting the effectiveness of this technique in sep-

Table 1. Optimum time/temperature regimes of accelerated aging (AA) for differentiating seed lot vigour within species.

Species	Hours of AA at 41°C
Slash pine	144
Longleaf pine	96
Loblolly pine	96
Pecan	144
Pecan (stratified)	96
Water oak	108

arating seed lots of varying degrees of vigour. Based on this experience, the optimum temperature for accelerated aging of tree seeds of these species appears to be 41°C.

Accelerated aging conditions that gave the best vigour differentiation between and among seed lots at 41°C varied by species (Table 1). Slash pine and pecan seeds appeared to withstand longer periods of aging than the other species investigated. Whether this has something to do with a tolerance of these species to higher temperature is not clearly demonstrated. However, the conditions in Table 1 are the most effective for differentiating relative seed vigor of these species.

Germinative Response to Accelerated Aging

Accelerated aging at 41°C and near 100% RH caused a decline in vigour (germination capacity) in all tree seed lots investigated regardless of vigour (Blanche et al. 1988a, 1988b, 1989; Matta and Blanche 1989; Marquez-Millano 1987). The rate of decline, however, varied with species and seed lots within species, thus making it possible to distinguish which seed lots are the most vigorous comparatively. In general, the pine seed lots declined in vigour much faster than the hardwood seed lots (Blanche et al. 1988a). Whether this is a characteristic response of pine seeds to AA is not entirely clear. An example of the response of tree seeds to AA is shown in Fig. 1 with three cultivars of pecan (Desirable, Schley, and Stuart). Although initially one of the cultivars (Schley = SLY) has a higher vigour, two of the cultivars are comparable. This demonstrates the possibility of detecting varietal differences in seed vigour.

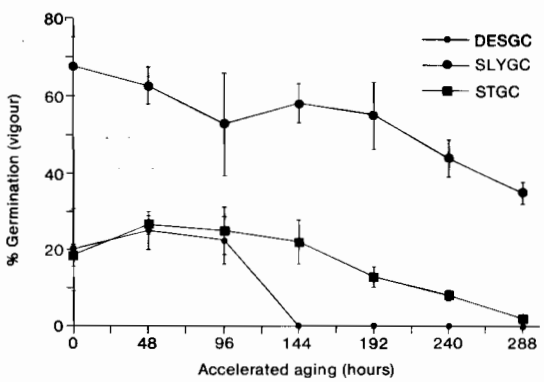

Fig. 1. Germinative response of pecan cultivars to accelerated aging.

Whereas accelerated aging functions mainly to rate relative vigour of seed lots and predict relative storability (Delouche and Baskin 1973), we have consistently observed that short periods of aging caused enhancement in germination (Table 2) (increased germination rate) especially with water oak and pecan seed lots (Blanche et al. 1988a). Such enhancement may be related to the increased level of hydration during the accelerated aging process, but other possibilities such as stimulation by high temperatures cannot be ruled out. This is being investigated further.

Chemical Changes During Aging

Chemical changes during accelerated aging have been reported earlier for water oak seed (Blanche et al. 1989). There was a gradual decline in starch during aging, while amino N dramatically increased. Both changes reflect the breakdown of polymeric compounds to their monomeric units. Reducing and nonreducing sugars exhibited no definite pattern of change, indicating the rapid interconversion of these forms of sugar. It is apparent that the increased level of metabolism during accelerated aging would result in the reduction of food reserves and therefore a consequent decline in seed vigour.

Changes in Moisture Content

All the seed lots subjected to accelerated aging increased in their moisture contents (Blanche et al. 1988a, 1988b; Matta and Blanche 1989). The rate of uptake of water during aging appears to be related to the initial moisture content of the seed and to the kind of food reserves present, particularly the lipids. a high proportion of lipid storage in the seed limits the uptake of water. Pecan seeds

Table 2. Germination values (GV) of pine and hardwood seeds subjected to different periods of accelerated aging (AA).

	Germination Values						
	Hours of accelerated aging						
Species	0	48	96	144	196	240	288
Longleaf	16.4	30.28	17.6	3.09	0.04	0	0
Slash Pine	40.72	39.26	24.82	1.44	0.09	.0065	0
Pecan	20.53	24.1	22.85	22.4	14.81	2.06	1.21
Water Oak	17.88	23(24)*	21(36)*	20(64)*	19(108)*	8(188)*	8.0

*Numbers in parentheses are the accelerated aging periods for that species.

contained a much higher percentage of lipids (44.5%) than longleaf pine seeds (25.2%), thus the stark difference in the rate of water uptake during accelerated aging (Fig. 2). This increased level of hydration of the seeds may be responsible for the enhancement of germination (increased germination rate) by short periods of accelerated aging (Table 2, Blanche et al. 1988a).

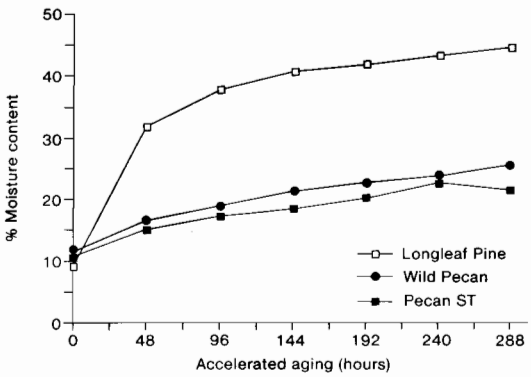

Fig. 2. Changes in moisture content during accelerated aging of pecan and longleaf pine seeds.

Leachate Conductivity During Accelerated Aging

There was an increasing level of leachate conductivity with increasing periods of accelerated aging (Fig. 3). This suggests some degree of membrane degradation or loss of integrity during AA. Because there was a high correlation between leachate conductivity and germination capacity (Fig. 4), seed vigour or quality can be assessed more quickly and simply using leachate conductivity, particularly under time-constrained situations. Bonner and Vozzo (1986) have earlier demonstrated the utility of leachate conductivity in estimating the quality of longleaf and loblolly pine seeds.

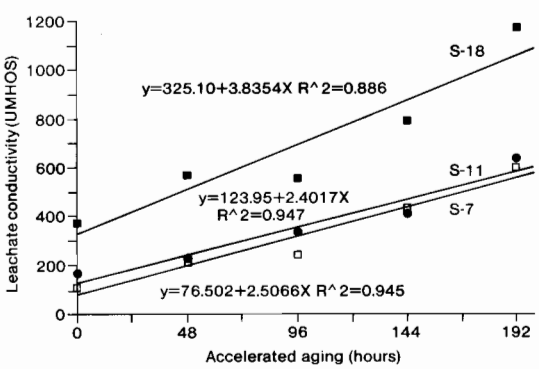

Fig. 3. Changes in leachate conductivity with increasing period of accelerated aging.

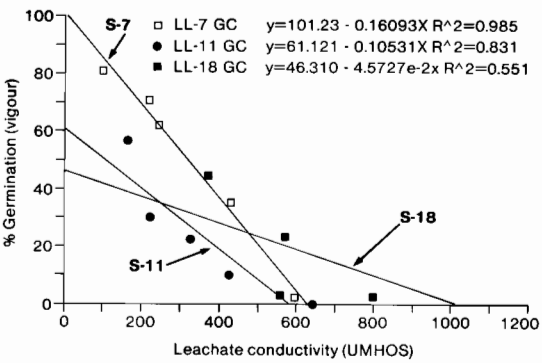

Fig. 4. The relationship between leachate conductivity and vigour (germination capacity).

Problems Associated with Accelerated Aging

The most annoying problem associated with the accelerated aging technique is the explosive growth of fungi for aging periods starting at 96 hours, in spite of very intensive seed sterilisation. In fact, at higher temperatures of aging, fungal growth starts even earlier (after 48 hours). Preliminary identification of the fungi revealed that most belong to the general *Aspergillus* and *Penicillium*. Initial attempts at resolving the problem by using fungicides, such as Captan, has met with limited success. Although there was a reduction in fungal growth, the fungicide caused abnormal germination. The compounding problem with a massive growth of fungi is the problem of determining the rotten seeds since these are evaluated in expressing germination capacity.

Another problem associated with accelerated aging is the interference from initial seed moisture content on results. Seeds with different initial moisture contents would give different AA results (Blanche et al. 1989; McDonald 1977).

Advantages of the Accelerated Aging Technique

In spite of these problems, the potential benefits of this technique are considerable. Moreover, it is inexpensive to establish and so simple to learn that no additional technical training is necessary. More importantly, it has been shown with some agricultural crops that results from this vigour test closely reflect emergence under field conditions.

The technique not only has the potential to predict relative seed storability and estimate vigour but the unexpected initial enhancement of germination presents another aspect in the study of the germination process. Therefore, its promise for testing vigour of tree seed should be seriously explored. We anticipate that this technique will work equally as well with seeds of multipurpose tree species as has been demonstrated with both

orthodox and recalcitrant seed in the temperate region.

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Evaluating Seed Quality of *Peltophorum pterocarpum* by X-radiography and Germination.

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Abstract

Based on x-radiographic images of seed development and the degree of decay, four anatomical classes can be recognised and reliably used for estimating the germination potential of *Peltophorum pterocarpum* Back.ex Heyne seeds. Germinability estimated by x-radiography compared equally well with the germination test results. Among all the seed pretreatments applied, the most effective and practical was soaking seeds in concentrated H_2SO_4 for 45 minutes.

SEED quality is usually evaluated by germination or viability tests as standardised by the International Seed Testing Association (1985). Usually, viability tests are faster than germination tests. At present, it is recognised that x-radiography is the quickest method for viability testing and is widely used in Europe and North America for forest tree seed research.

Kamra (1976) indicated that for tropical forest trees x-radiography could be reliably used to rapidly detect the content of a fruit, and the degree of seed development, mechanical damage, insect attack, disease, etc. He also discussed the different applications of x-radiography in tropical seed collections, and in processing and storing seed. An attempt to introduce this method to tropical countries was made by the IUFRO Working Group on Seed Problems in 1984, when a special training course on x-radiography was held at an IUFRO Symposium on Seed Problems in Bangkok, Thailand. Subsequent research work at the ASEAN-Canada Forest Tree Seed Centre has confirmed that x-radiography can be effectively applied to testing of tropical forest tree seed.

The present paper reports on experiments designed to determine anatomical seed development classes for evaluating the germination potential of *Peltophorum pterocarpum*. It also discusses effective pretreatments for overcoming seedcoat dormancy and for achieving maximum germination of this species.

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Materials and Methods

Seeds of *P. pterocarpum* for this study were collected from Pakchong District, Nakhon Ratchasima Province, Thailand (lat. 14° 38′N, long. 101° 22′E, elev. 390 m.) in September 1987. They were stored in a cold room at 2°C before use. The seed moisture content, determined by the oven method at 103 ± 2°C for 24 hours, was 8% (fresh weight).

The work was carried out at the National Tree Seed Centre, Petawawa National Forestry Institute, Chalk River, Ontario, Canada and involved three sets of experiments.

Seed pretreatments

The following pretreatments were applied to the seed:

A) Soaked in 85°C water and left for 24 hours in the water as it cooled to room temperature (20°C);

B) Cut off < 1 mm of seedcoat at the embryo end;

C) Cut off < 1 mm of seedcoat at the embryo end and then soaked in water at ambient temperature for 24 hours;

D) Cut off 2–3 mm at the cotyledon end;

E) Cut off 2–3 mm at the cotyledon end and then soaked in water at ambient temperature for 24 hours;

F) Soaked in concentrated H_2SO_4 (1.84 specific gravity) for 15 minutes and then rinsed with tap water for 10 minutes;

G) Soaked in concentrated H_2SO_4 (1.84 specific gravity) for 45 minutes and then rinsed with tap water for 10 minutes;

H) Control (no treatment).

Four replications of 25 seeds each were used for each treatment. Both treated and untreated seeds were sown onto a Kimpak germination medium in Petawawa germination boxes (Wang and Ackerman 1983). The Kimpaks were moistened with 125 ml of distilled water; another 125 ml of distilled water was placed in the water reservoir of the box. The prepared seeds were germinated in Conviron G30 Germination Cabinets (Conviron Products Company, Winnipeg, Canada) at 30°C constant temperature, 85% relative humidity, and 8-hour illumination from cool white fluorescent lights of 1400 lux. A seed was considered germinated when its radicle was at least equal to the seed length and the seed coat was partly shed. Germination progress was observed every day and lasted for 21 days after sowing.

Anatomical Classification of Seed Development from X-radiographs

X-radiographs were taken with a Faxitron x-ray system, model 43807N (Hewlett Packard), on Kodak Industrex M x-ray films. Seeds were x-rayed at 17.5 kV, 3 mA, 60 seconds, and 56 cm focus-film-distance.

Approximately 5000 seeds were x-radiographed as described above; their radiographic images were studied with a screen viewer to evaluate seed structure and to classify the degree of embryo and cotyledon development. Microscopic examinations of dissected seeds were also made. Four classes of anatomical development of the seeds (Fig. 1), based on their embryo and cotyledon development and degree of decay, were recognised. Classified seeds were separated and germinated in the manner described above. Seeds classified from their x-radiographs were pretreated with the most effective method for germination.

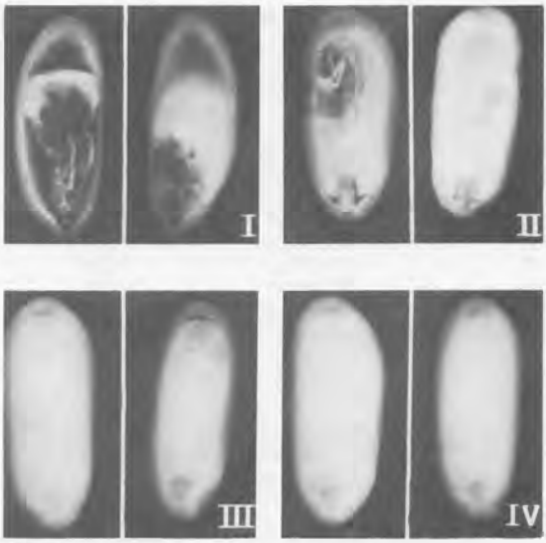

Fig. 1. Anatomical seed development classes by x-radiography

Class I	embryo	: none, completely deformed or shrunken
	cotyledons	: completely deformed or shrunken
Class II	embryo	: partly deformed or shrunken
	cotyledons	: partly deformed, shrunken or decayed
Class III	embryo	: clear vascular bundle and regular density
	cotyledons	: irregular density, blackened spot or mechanical damage
Class IV	embryo	: clear vascular bundle and regular density
	cotyledons	: regular density

Comparison of Predicted Seed Germinability by X-radiography with Germination Testing

To verify the accuracy of classification of seeds by x-radiography, eight samples of 100 seeds each were x-rayed, pretreated, and germinated. Only germination data from 7 days after sowing were subjected to statistical analysis (Table 1). The x-radiographic pictures were evaluated for germination potential and compared with germination test results.

Results and Discussion

Seed Pretreatments

Results of the seed pretreatment are shown in Table 1. Seven days after sowing the number of hard, ungerminated seeds remained high with pretreatments H, A, and F. In an earlier study by Kobmoo and Hellum (1984), soaking in conc. H_2SO_4 for 15 min. and mechanical scarification were found to be most effective in overcoming

Table 1. Effects of pretreatments on germination of *P. pterocarpum* seeds.

Pretreatment	Germination percentage	
	7 days after sowing in germinator	21 days after sowing in germinator
A	66.00 c*	72.00
B	82.00 c	82.00
C	79.00 cd	79.00
D	87.00 d	87.00
E	79.00 cd	79.00
F	36.00 b	70.00
G	86.00 d	86.00
H	7.00 a	48.00

*Germination comparisons followed by the same letter are not significantly different at $p = 0.05$ according to Duncan's New Multiple Range Test (Sokol and Rohlf 1969).

seedcoat dormancy and obtaining maximum germination of *Cassia siamea* Britt. seeds. Pretreatment by cutting off a small piece from the seeds at the radicle or cotyledon ends (pretreatments B and D) and soaking seeds in conc. H_2SO_4 for 45 minutes (pretreatment G) resulted in complete and rapid germination of *P. pterocarpum* seeds (Fig. 2). However, we obtained our best results from pretreatment by soaking in conc. H_2SO_4 for 45 minutes (G) or by cutting the end of the cotyledon (D). Properly acid-scarified seeds exhibited swelling and dull, but not deeply pitted, seedcoats in agreement with observations by Bonner et al. (1974). Although pretreatment B gave good results, it should be applied with caution, because some abnormally germinated seeds were observed, caused apparently by cutting injury to the embryo. It is possible that the decreased germination rates of pretreatments C and E, which involve soaking, as compared to those of B and D, were caused by excessive hydration.

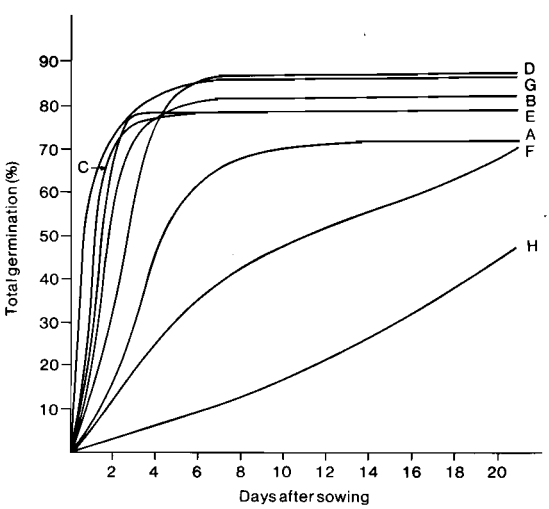

Fig. 2. Germination courses of *P. pterocarpum* seeds by different pretreatments.

As shown in Fig. 2, it took 21 or more days for the control to reach maximum germination. On the other hand, the most effectively treated seeds started germinating 3 days after sowing and completed germination within 7 days. Considering this pattern, it is reasonable to state that a proper germination test for this species can be achieved in 7 days. An overall comparison of the germination percentage, speed of germination, and practicality suggests that soaking in conc. H_2SO_4 for 45 minutes and rinsing with tap water for 10 minutes (G) was the most effective pretreatment for *P. pterocarpum* seeds. Therefore, we used this pretreatment for comparison with germinability assessments by x-radiography.

Anatomical Classification of Seed Development from X-radiographs

Fig. 3 shows the major anatomical structures of *P. pterocarpum* seed, seedcoat, embryo, and cotyledons. According to x-radiographs of seed structure and degree of development, the seeds were identified as shown in Fig. 1. Seed structure of *P. pterocarpum* appears to be similar to type A for broad-leaved species as described by Simak (1980), although his seed development classification does not correspond with all categories observed in our experiments.

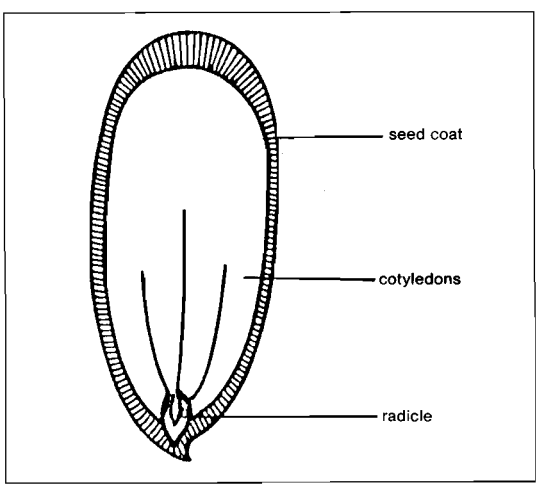

Fig. 3. The internal structure of *P. pterocarpum* seed.

Table 2 shows the germination percentage of seeds in the different anatomical classes as determined by x-radiographs. The germination

Table 2. Germination results of anatomically classified *P. pterocarpum* seed (by x-radiography) 7 days after sowing.

Classes	% germination		
	Normal	Abnormal	Dead
I	0.00 c*	3.00	97.00
II	32.67 b	46.00	21.33
III	94.67 a	2.67	2.67
IV	97.33 a	1.33	1.33

*Germination comparisons followed by the same letter are not significantly different at $p = 0.05$ according to Duncan's New Multiple Range Test.

capacities correspond with seed classes in an ascending order from I to IV. It was therefore confirmed that classification by seed structure and development using x-radiography as described above is accurate and can be used for estimating seed germination potential for this species.

Comparison of Predicted Seed Germinability by X-radiography with Germination Testing.

The comparative germinability assessment by x-radiography and germination test results is shown in Table 3. A paired t-test (Sokol and Rohlf 1969) indicated that there was no significant difference between both tests at $p = 0.05$. It is clear then that x-radiography can be used to rapidly and efficiently evaluate seed germinability of *P. pterocarpum*.

Table 3. Comparison of seed germinability percentage: x-radiography and the germination test.

Sample no.	Germinability by x-radiography (%)	Germinability by germination test (%)
1	85.00	85.00
2	82.00	79.00
3	79.00	81.00
4	87.00	84.00
5	79.00	81.00
6	87.00	86.00
7	86.00	85.00
8	87.00	85.00
Mean	84.00	83.25

Conclusions

According to the results obtained from these experiments, it can be concluded that:

1. Based on x-radiographs, seed quality of *P. pterocarpum* can be grouped into four classes according to x-ray images of their anatomical development.
2. Seedcoat dormancy of *P. pterocarpum* is best released by H_2SO_4 scarification or by cutting 2-3 mm of the seedcoat from the cotyledon end. However, the former method may be preferred because it is easy to do.
3. X-radiography can be used to predict germinability in this species.

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Testing of Subtropical and Tropical Forest Tree Seeds by X-radiography

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Abstract

The paper describes three different principles useful for testing subtropical and tropical forest tree seeds by x-radiography. These principles are X-test, XC-test and XI-test. The direct radiography test (X-test) reveals the internal structure of seeds, i.e. shows whether the seeds are filled, empty, insect-infested, poorly, well or abnormally developed, etc. In the XC-test the seeds are treated with aqueous or vaporous contrast agents in order to get a more informative radiographic image on seed viability. An XI-test reveals the viability of the seeds at an early stage of incubation. All these tests serve as a basis for practical measures to be taken in connection with collection, processing, particularly cleaning, storage, trade and conditioning of the seeds for sowing in nursery.

EARLY attempts to use x-rays for distinguishing between empty and filled seeds of Scots pine (Hermelin 1904) were not successful, because at that time the x-ray technique was not fully developed for studies of this kind. Later, x-radiography was used to detect insect infestation in agricultural seeds. Since the beginning of the 1950s, radiography has been used permanently in research and testing of forest tree seeds as is evident from the extensive literature published in this matter (cf. Simak and Sahlén 1983). At present, testing of seeds using radiography is performed according to the following three principles:

(a) Determination of internal structure of seeds by direct radiography (X-test)
(b) Determination of seed anatomy, viability and mechanical damage using contrast agents (XC-test)
(c) Radiography of seeds during their physiological activity (XI-test).

In this presentation the above principles are briefly described as well as suggestions how to apply them to subtropical and tropical forest seeds. In the paper, reports from workshops held in Mexico 1980 (Simak 1981b), Thailand 1984 (Simak, in press), and China 1985 (Simak 1986) are often referred to.

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X-test

The internal structure of seeds showing whether a seed is empty, filled or insect-infested can be studied by cutting or dissection test (C-test), or by x-radiography (X-test). In X-test the seeds (defined as fruits used as sowing units for the purpose of this paper) are radiographed dry, without any pretreatment except that wings and other appendages that could have a disturbing effect on the quality of the radiographic image are removed. Concerning the efficiency of C- and X-tests, it was shown that X-test is mostly quicker and easier to perform and interpret than C-test.

The great advantage of X-test is that it is non-destructive and therefore the seed replicates analysed by X-test can be used in a subsequent germination test as well. This is of course not possible with the seeds that were used for C-test and therefore the germination test must be performed on a parallel seed sample (cf. the prescriptions of ISTA, 1985). The frequency of empty seeds can vary considerably among replicates of a sample

(Gordon and Wakeman 1978). Thus, the germination values calculated on the basis of filled seeds determined indirectly by C-test are less reliable than those based on X-test. Another advantage is that radiographs can be filed and reanalysed at any time, if desired. The superiority of the X-test for determination of empty and insect-infested seeds was recognised by ISTA (1985) which included the X-test in its prescriptions (5.6.5.A.3) as an alternative method to the cutting test.

X-test seems to be a great help for analysing the internal structure of tropical seeds (Kamra 1974, 1976). Comparative studies between X- and C-tests were carried out on several tropical seeds at the workshops in Mexico and Thailand, particularly on *Pinus ayacahuite, Pinus patula, Pinus caribaea, Abies religiosa* and *Bischofia javanensis*. The result obtained by X-test was more reliable than that produced by C-test. For some small seeds such as *Cordia alliodora* it was practically impossible to interpret C-test satisfactorily while an experienced analyst would easily recognise the empty, insect-infested or filled seeds of this species on a radiograph (Fig. 3, Simak 1981b). However, there are also tree species with big-sized fruits or seeds and with complicated structures in coat, pericarp or embryo for which the X-analysis is less suitable.

Filled seeds of a species sometimes show a great variation in their anatomical development. This can occur both for coniferous and broad-leaved trees, especially on the border of the natural distribution area of a species. Seed workers in northern Scandinavia observed very early that there is a relation between anatomical seed development, climate and seed germination. In this connection, tests have been developed in which the seeds of *Pinus silvestris* are split by cutting and the lengths of the embryos and gametophytes are measured under microscope. On the basis of the figures obtained for the relations between embryo and gametophyte lengths, the germinability of the seeds can be determined (Wilbeck 1930).

Besides the fact that such a procedure is time-consuming, it must be pointed out that the correlation between embryo development and germinability is determined indirectly, because the C-tested seeds are destroyed and therefore the germination test has to be made on a separate sample. Using X-test, the above parameters can be estimated on the radiograph, which makes the tedious analyses under microscope superfluous. Moreover, thanks to the fact that X-test is non-destructive, the relationship between embryo development and germinability can be established directly on the same seed sample. In northern countries, where variations in embryo development are common due to the harsh climate, X-test has become routine for determination of the anatomical potential of coniferous seeds to germinate (Simak 1980). The embryo development in hardwood seeds was extensively studied in the Soviet Union (Smirnova 1978).

Poorly developed seeds have been observed also in subtropical and tropical countries though the climate there differs from that in northern countries. Thus, in a sample of *Pinus caribaea* from Cuba it was found that 14% of the seeds had poorly developed embryos and gametophytes with a germinability between 0 and 40%. Fully developed seeds in the same sample showed a germination percentage of 95%. These variations in seed anatomy were most probably related to environmental conditions. Unfortunately, the history of the sample was not documented (Simak, unpubl.). Unproductive seeds such as those in the *Pinus caribaea* sample can be removed from the bulk by different methods. Such conditioning of seeds can be very effective if conducted under X-test control.

Thus, Bergsten and Sundberg (these proceedings) removed poorly developed seeds of *Cupressus lusitanica* by flotation in water in specially constructed equipment, and controlled the process by X-test. The germinability of the seeds increased most considerably — from 20 to 60%. The tropical Mexican *Pinus ayacahuite* produces seeds with varying embryo length as a consequence of the course inherent in the embryogeny specific for this species. Thus, a sample analysed at the workshop in Mexico had the following embryo spectrum according to X-test:

Empty seeds	20%
Seeds with embryo shorter than 50% of the embryo cavity	20%
Seeds with embryo length between 50 and 75% of the embryo cavity	28%
Seeds with embryo longer than 75% of the embryo cavity	32%

With adequate conditioning of this type of seeds, their germinability can increase considerably. The treatment is usually adjusted to the degree of embryo development.

It would be of practical interest to obtain information about the variations in seed anatomy within the different subtropical and tropical tree and shrub species, as these variations mostly correlate with seed quality. For this purpose, the X-test is extremely useful.

XC-Test

X-ray contrast method requires that the seeds to be radiographed are treated with chemicals that

increase the contrast of certain seed tissues on the radiograph. The tissues concerned are as follows: 1. physiologically dead tissues; 2. specific tissues in living seeds; 3. mechanically damaged tissues. The contrast agents are applied in aqueous or vaporous states.

1. The most frequently used contrast agents are heavy atomic salts such as $BaCl_2$, NaI, KI in aqueous solutions. The principle (for $BaCl_2$) is that the agent by diffusion penetrates only into the necrotic (or damaged) parts of the seeds. The tissues impregnated in this way appear then on the radiograph in clear contrast to the healthy tissues owing to the strong absorption of x-rays by the heavy atoms of the contrast agents. However, other principles can be in force if other chemicals are used. The viability of a seed can be estimated from the size of the necrotic (impregnated) area in a seed. The method was developed for *Pinus silvestris* (Simak 1957), *Picea abies* (Kamra 1971), and for a broad-leaved tree, *Fraxinus excelsior* (Machanicek 1976). The XC-method as a viability test was also successfully applied to some seeds of tropical coniferous trees such as *Pinus caribaea, Pinus patula* (Kamra 1981), *Pinus kesiya, Pinus merkusii* (Kamra 1984), *Pinus oocarpa* (Kamra 1985) and *Pinus elliottii* (Kamra 1987). Sodium iodide in aqueous solution was mostly used as contrast agent.

2. Some chemicals in aqueous solution show specific affinity for different tissues, both dead and living ones, in *Juglans* seeds (Vozzo 1978). How these contrast agents work in living tissues is unknown. Nevertheless, the method can be of interest for anatomical studies of big-sized tropical seeds and fruits with complicated anatomy. Tomography may be an additional improvement in such studies (Vozzo 1974).

3. Mechanical damage to seeds of *Pinus silvestris* can be detected by XC-test using aqueous contrast agents (Simak 1957; Kamra 1963). Recently, certain chemicals in vaporous state, e.g. iodine, have been used for this purpose. Not only iodine but also chemicals of lower atomic weight such as $CHCl_3$ can give excellent contrast, since the relative absorption of soft x-rays by the elements is distinctly different from that of hard x-rays (Simak 1974b). For example, by using soft x-rays one can get a high contrast between the non-impregnated undamaged seed tissues containing mainly H, O, C, and the damaged tissues penetrated by $CHCl_3$-vapours despite the relatively low atomic weight of the chlorine. (Atomic weight: H = 1, C = 12, O = 16, Cl = 35).

Chloroform and other halogen derivatives of alkanes are very sensitive agents, indicating even extremely slight internal damage to seeds. Preliminary studies at our laboratory showed that it is the fat in the damaged seed tissues that is attacked by the chloroform vapours and thereby becomes impregnated. This fat-principle sets limits to the use of chloroform XC-test only on seeds with high fat content, e.g. coniferous seeds. The method was developed for *Pinus silvestris* (Simak 1974a).

The sensitivity of this test can be demonstrated on seeds of this species by dropping them a few times on to a hard surface. After such treatment no damage will be observable even if the seeds are studied under microscope. However, if the same seeds are exposed to chloroform vapours for 4 hours and then radiographed, the internal damage to the seeds caused by the dropping will appear clearly on the radiograph as small, impregnated, round patches (dots) in the gametophyte.

In practice, such damage can occur for instance by machine-sowing (Bergsten 1980b) at which the seeds are exposed to impacts. The heavier the damage is, the greater and more frequent are the patches. Seeds which are heavily damaged, e.g. due to hard extraction and dewinging, can be totally impregnated. Slight damage (small dots) does not necessarily lower the germinability and vigour of the seeds if the germination is carried out immediately under optimal conditions in a laboratory. However, the germinability of these seeds will decrease under stress conditions, e.g. at high temperatures (Bergsten 1980a) or after a long-term storage. In Sweden the XC-test is used as routine for local certification of seed quality. Recently, the test was improved by combining it with the so-called PREVAC-test (Simak and Pehap 1987).

The chloroform XC-test was demonstrated on artificially damaged seeds of *Pinus caribaea* at the workshop in Mexico and on *Pinus kesiya* seeds at the workshop in China. Ocular investigations of the damage using magnifier or microscope gave very unsatisfactory results while the vaporous XC-test on the same seeds was very convincing. During handling and processing, the tropical seeds are often mechanically damaged to a great extent. This leads not only to economical losses and waste of seed material but also renders seeds vulnerable to infections by fungi, especially in storage, etc. Through inspection of the different steps in the processing technique using vaporous X-test, the source(s) of the damage can be revealed and eliminated. Thus, the quality of the seeds produced can be improved. The heavily mechanically damaged seeds can be removed from a bulk using the PREVAC-method (Bergsten and Wiklund 1987).

XI-Tests

By direct X-test and XC-test the seeds are analysed in the state of anabiosis. However, the

seeds can also be radiographed at a stage of high biological activity, for instance at the very first stages of the germination process, before the protrusion of the radicle from the seed (X-incubation tests; XI-tests). Hereby information can be obtained about the viability, vigour, abnormal germination, etc of the seeds.

The so-called IDX-test for a quick determination of seed viability is based on the principle that viable seeds possess a higher ability than dead ones to retain imbibed water when dried (I = incubation for 3 days at 15°C, D = drying for 2-4 hours depending on ambient conditions, X = x-radiography of the seeds). The different levels of water in the viable and dead seeds after the drying step is clearly distinguishable on the contrast of the radiographic image. The method was first used on *Pinus contorta* (Simak 1984b), but it is likely that the process will be similar for other species, too. IDX-test is non-destructive, i.e. seeds with different ability to retain water can be selected by x-radiography and be individually germinated. The viability of the seeds can then be correlated with the water absorption pattern on the radiograph.

Thus the criteria for IDX-test can be directly established. Such a procedure is not possible if chemicals in aqueous agents are used. These are toxic and kill the seeds, particularly those with low vigour. The x-ray dose used by radiography of the activated seeds (seeds with high mc) is very low and does not influence seed germination (Simak and Dirke, unpubl.).

At the workshop in Thailand 24 students interpreted radiographs of 150 seeds of *Pinus merkusii* tested by IDX-method according to the individual germination analysis of the same seeds; 45 out of 150 seeds germinated, 93 were dead and 12 were empty. Though the students were not experienced in interpreting radiographs, a few minutes explanation about the IDX identification was enough for 21 out of the 24 students to identify correctly 98% or more of the 150 seeds in respect of their viability.

Additionally, it can be mentioned that the ability of viable seeds to retain imbibed water is so distinct that the above principle is also used in practice for separation of viable seeds from dead ones in a bulk. After incubation and adequate drying, viable seeds with their high density can be easily separated from dead seeds by flotation in water — the so-called IDS-method (Simak 1981a, Bergsten 1987). In Sweden, this separation is used routinely on several 1000 kg lots of seed yearly. In Ethiopia, for instance, the quality of *Pinus patula* has been notably improved by this method though the separation was carried out using rather simple facilities (Örlander 1986).

An improvement of seed quality by using the IDS-method was also obtained for *Pinus caribaea* and *Pinus oocarpa* (Simak 1984a) and *Cupressus lusitanica* (Bergsten and Sundberg, these proceedings). Thus the successful IDS-separation itself confirms the practical applicability of IDX-test.

In seed testing, ungerminated seeds at the end of a germination test (UE-seeds) should be estimated as viable or dead by a cutting test, (ISTA 1985). The viable and the dead UE-seeds can also be easily distinguished by an XI-test performed on the same principles as the IDX-test, as was shown for *Pinus silvestris* (Simak et al. 1989). For UE-seeds, the incubation step is omitted and the dead seeds are usually more decayed (sometimes empty), being incubated in the germinator for 21 days. For practice, adequate information about the quality of UE-seeds in a germination test is of great importance. The reason for the occurrence of fresh UE-seeds can be dormancy or low vigour of seeds. A good knowledge of the quality of UE-seeds enables the proper measures for seed conditioning, i.e. stratification, removal of dead seeds, etc. to be taken.

At the workshop in Thailand most of the 36 participants testing the UE-seeds of *Pinus kesiya* and *Pinus merkusii* by x-radiography produced good results. Likewise, at an international ISTA-project dealing with the determination of UE-seeds, the above two species were tested with good results. Moreover, the UE-seeds of *Pinus kesiya* showing by cutting test viable gametophytes and dark green, swollen embryos not capable of penetrating the seed coat could also be identified on the radiograph. The embryos of these abnormal seeds showed a high absorption of x-rays.

There seem to be several possibilities for studying the biological processes in the activated seeds with x-radiography, which can benefit seed research and testing. Undoubtedly, XI-tests are worth being further investigated.

Conclusions

Applications of X-Radiography in Seed Testing

- Purity: Different types of sample components that look like seeds can be identified.
- Sampling: Samples (replicates) can be checked for accuracy; e.g. distribution of empty seeds. Discrepancies among the different sampling instruments can be revealed.
- Seed damage: Insect and mechanical damage to seeds can be identified. This concerns also damage invisible to ocular inspection.
- Germination test: The evaluation of the germination test on the basis of filled seeds can be made more exactly by X-test than by C-test.
- Seed vigour: Anatomical seed development can

be determined accurately and thereby the seed vigour can be estimated.

Practical Measures Based on Different X-Tests

- Collection of poorly developed seeds can be avoided.
- Cleaning of the seeds can be controlled effectively.
- Steps in the processing technique causing damage to the seeds can be revealed.
- Amount of unproductive seeds can be reduced to a minimum.
- Conditioning of seeds can be performed purposefully.
- Sowing density in the nursery can be properly adjusted.
- Insect control in international trade can be facilitated.
- Price setting of a seed lot can be done more reliably.

Principles for Seed Testing Using X-Radiography

Test Principles	X-Test n-d	XC-Test d	XI-Test n-d
The test concerns:	internal structure of s. (anatomy & defects)	damage to the tissues (physiological & mechanical)	physiological reactions of viable and dead s.
S. are radiographed in state of:	anabiosis	anabiosis	biological activity
Treatment of s. prior to radiography:	none	aqueous & vaporous contrast agents (CA)	incubation at different conditions
Principles:	direct radiography	selective penetration of CA into the tissues	different water dynamics in viable and dead seeds
Alternative tests:	cutting and dissection d	vital staining d	chemical and physical d

d = destructive test **n-d** = non-destructive test **s** = seed

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High-Voltage Electron Microscopy of Cell Walls in *Pinus taeda* Seeds

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Abstract

Observations by high-voltage electron microscopy (HVEM) relate germination potential of loblolly pine seeds to their cell walls. Seed germination can be predicted by measuring the conductance in deionised water resulting from seed leachate. HVEM micrographs link traumatised cell walls in seeds with greater measured conductance and intact cell walls with little conductance.

RECENT investigations report relationships between viability of pine seeds and electrical conductance of leachates in deionised water after prescribed soaking (Bonner and Vozzo 1982, 1986; Vozzo 1984; Vozzo and Bonner 1985). As pine seeds lose their ability to germinate readily, more leachate is released into the deionised water, which is revealed by greater conductance. Leachate of a good seed then will conduct less current than leachate of a poorer seed. What is the mechanism or disorganisation sequence that permits more leachate escape from less viable seeds?

Woodstock (1983) discussed potassium-ion loss through seed coats during normal germination processes of soybean seeds. However, he indicated a point where deterioration of the seed resulted in a loss of growth potential, which can be correlated with electrical conductance of leachates. Murphy and Noland (1982) also reported that temperature effects on seed imbibition are mediated by membranes and resultant leakage. Specifically, Woodstock (1986) cites the lipid-protein membrane of cell walls as an agent in ion transport in soybean seeds.

Pine seeds are also subject to these physiological challenges. We have adapted ultrastructural observations to this relatively new procedure in seed research to determine if membrane trauma is involved in electrolyte release from pine seeds.

Use of the New York State High-Voltage Electron Microscope (HVEM) was assisted by PHS grant no. RR01219, awarded by the Division of Research Resources, DHHS. The HVEM offers considerable range with enhanced resolution and definition over conventional electron microscopes. The study reported here uses HVEM findings to relate seed membrane trauma to resulting leachate escape.

Materials and Methods

Loblolly pine seeds (*Pinus taeda* L.) were collected fresh and stored at 3°C. Prior to leachate evaluation by electrical conductance, seeds were soaked in water for 16 hours, surface dried, and placed in chambers with deionised water for conductance determinations using an ASAC-1000. For HVEM observations, seeds were simply divided into lots of high conductance (greater than 75 A are poor seeds) and low conductance (less than 65 A are good seeds).

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Specimens from high- and low-conductance samples were prepared for HVEM by EMLABS, Inc., of Birmingham, AL. Tissues were fixed in cold buffered 2% glutaraldehyde, post-fixed in 1% cold buffered osmium tetroxide, and dehydrated in graded ethanols and then in propylene oxide. The specimens were embedded in freshly catalysed Poly/Bed 812 resin (Polysciences). Thick sections (0.5–1.0 mm) were cut with glass knives on the LKB Ultrotome III and mounted individually on Formvar-coated slot grids. Grids were stained in 2% aqueous uranyl acetate at 50°C for 1–4 hours according to thickness, followed by 30–45 min. in Reynolds lead citrate at room temperature. The AEI EM7 Mk II 1.2 MV HVEM (NIH Biotechnology HVEM Resource in Albany) was used at an acceleration voltage of 1.0 MV to obtain stereo views of the sections (King 1981).

Results and Conclusions

Fig. 1 illustrates a typical undifferentiated parenchyma cell found in loblolly pine seeds. Note that it is vacuolated and rich in storage granules. The primary distinction between cells of seeds with low conductance and those with high conductance is the integrity of the cell walls. Fig. 1 shows (arrow) a traumatised cell wall with a complete break in the entire cell wall structure (plasma membrane, middle lamella), which is characteristic of a seed producing high conductance in deionised water.

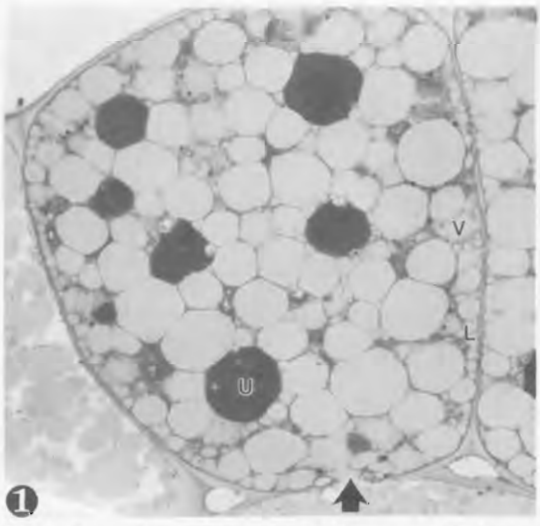

Fig. 1 Parenchyma cell from loblolly pine seed. Arrow identifies trauma in cell wall; V, vacuole; L, lipid storage granule; U. uranylophilic dense areas. Uranyl and lead stain. ×7500.

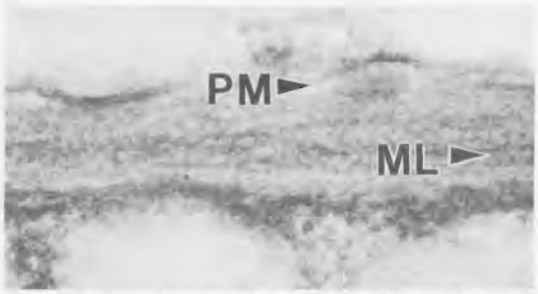

Fig. 2 Portion of cell wall from a normal cell of loblolly pine seed. PM, plasma membrane; ML, middle lamella. Uranyl and lead stain. ×140,000.

Seeds with leachates of lower conductivity have cells with intact cell walls (Fig. 2). The plasma membrane (even though only 80 A wide here) of the intact cell wall shown in Fig. 2 limits passage, both active and passive, into or out of the cytoplasm. Compare its intact anatomy with a broken cell wall (Fig. 3). Fig. 3 is a stereo-pair and may be viewed in three dimensions using a stereo viewer. Dissolution of the plasma membrane allows free passage from cytoplasm through the normally selectively-restraining cell wall.

Fig. 3 Stereo-pair of section from broken cell wall (arrow) of loblolly pine seed. Uranyl and lead stain. ×65,000, ± 10° tilt.

We found a consistent difference in cell-wall integrity between seeds having high and low conductance. It is known that the trilaminar plasma membrane affects ion passage by confining cytoplasm of adjacent cells (Ledbetter and Porter 1970).

Similarly, localised damage by temperature, moisture content, or pathology to the unit membrane structure of other organelles (plastids, mitochondria, dictyosomes, nuclear envelope) may also contribute to unorganised, nonselective leaching.

Current research continues to identify the leachate constituents from seed samples.

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Seed Processing and Storage

Methodology and Costs for Treatment of *Parkia biglobosa* Fruits

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Abstract

Basic calculations have been carried out at the Centre Nationale de Semences Forestieres (CNSF) in order to determine the pay for labourers when treating fruits of *Parkia biglobosa* in order to obtain seeds. On average 5 kg of fruits are needed to get 1 kg of seeds. But considerable differences have been noticed in the ratio fruits: seeds for the 17 samples studied. These differences are due to natural variation. So calculations for payment should not be based on the net weight of seeds obtained, but on the number of kg of fruits treated.

THE fruits from most forest species must be processed to extract seeds which are of easy and practical use — mainly for the nurseryman. This is the case for *Parkia biglobosa*. The extraction of *P. biglobosa* seeds demands the application of new techniques. Similarly, when paid workers are employed to do the work, it is important to define worthwhile specifications (for the worker and for the employer) in order to minimise the labour cost.

Preparation Techniques

Extracting seeds from *Parkia biglobosa* fruits involves separating the seeds from the fruit tissues surrounding them. A combination of several operations dictated by the nature of the *P. biglobosa* fruits is required.

Description of the Fruit

The *P. biglobosa* fruit is a thick indehiscent pod, which is brown in colour when mature. It contains seeds enveloped in a yellow pulp when ripe (Aubreville 1950; Bonkoungou 1986). This pulp is surrounded by two valves joined together.

Drying the fruits makes the pulp, which constitutes the mesocarp, crumbly and powdery. According to Busson (1965) the mesocarp represents 39% of the weight of the fruit. Moreover, the mesocarp has a high energising power as it contains more than 60% sugar (FAO 1967 — quoted by Booth and Wickens 1988).

Extraction of Seeds

Seed extraction is carried out in 8 successive stages; each one is as important as the other. These are shelling, predrying, pounding, winnowing or sieving, washing in water, sorting through flotation in water, drying, and visual sorting by hand.

The fruit is opened by separating the two valves, and seeds enveloped in pulp are obtained. This mixture is dried in the sun for a time varying according to its moisture content and the intensity of the sun. The seed is separated from the crumbly pulp by pounding (in a mortar, for example). The seeds in most cases retain their endocarp and a thin layer of pulp.

Depending on the equipment available, the seeds are then winnowed or sieved to separate the pulp from the 'seeds'. This yellow pulp is generally collected for human or animal (especially pig) feed.

Washing in water then assists in removing the pulp and thus extricates the 'seeds' from the remaining pulp and sometimes from the endocarp. If the endorcarp is removed during the wash-

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ing, pure seeds are obtained. At the same time as the washing, sorting by floating in water enables collection of the heavy seeds and all other heavy particles that sink to the bottom of the receptacle. The floating elements, including seeds and any other light particles, are thrown away. The seeds are spread in thin layers under an airy shelter and regularly shaken to give uniform drying.

A final operation increases the purity of the seed lots by manually removing spoilt seeds and heavy particles. The seeds thus obtained are tested, stored and despatched to the users. This stage is not required if the seeds are free from impurities after the washing process.

Determining the Specifications

Method

Seventeen fruitlots of *Parkia biglobosa* were weighed and given to 17 different workers.

For each worker, the time taken to do the following three operations for the extraction of seeds was noted: pulp removing, pounding in water, sieving, then sorting by hand. At the end of the extraction, the 17 seedlots obtained were weighed.

Results

1. Amount of fruit processed per man-day Table 1 shows, for the 17 lots, the weights (in kg) of fruits processed and the weights of the seed obtained over 8 hours (one man-day). The time taken in the three stages to process 1 kg of *P. biglobosa* is given in Table 2.

2. Fruit seeds : fruit weights ratio The ratio of seed weights to fruit weights for the 17 seedlots is presented in Table 1. Since a kg of treated fruits gives about 0.2 kg of seeds, it can be considered that 1 kg of seeds represents an approximate cost (in labour for the preparation) of 435 F CFA. As well as labour cost, the general processing cost of seeds would include amortisation of extraction equipment, cost of water, and supervision costs.

3. Quantity of fruits per man-day In Table 1 the mean quantity of fruits treated per man-day has been calculated at 11.8 kg and the standard deviation (S.D.) at 1.743. The range of the mean quantity of fruits treated per man-day : 11.8 ± 3.4 kg (= 8.4–15.2 kg) has been established at the 5% level of significance. Sample 596 did not fit in this range and was considered an extreme case.

Means and S.D. were recalculated for all data except for sample 596 (Table 1) and a more real-

Table 1 Weights of fruits and seed treated per man-day and the ratio weight of seeds: fruits.

Sample No.	Quantity of fruit (kg) treated per man-day	Quantity of seeds (kg) obtained per man-day	Ratio weight seeds: weight fruits
596	6.24	1.17	0.19
598	12.75	2.56	0.20
599	12.76	2.80	0.22
600	11.22	2.35	0.21
601	12.96	2.75	0.21
602	12.51	2.71	0.22
603	12.90	4.92	0.41
604	12.29	2.46	0.20
605	11.01	1.90	0.17
607	12.91	2.22	0.17
610	12.16	1.85	0.15
611	12.36	2.05	0.17
612	11.13	1.45	0.13
613	11.43	2.67	0.23
614	13.38	2.50	0.19
615	9.38	2.50	0.19
616	14.07	2.80	0.20
Mean	11.81	2.41	
S.D.	1.743	0.79	
— Sample 596			
Mean	12.15	2.49	0.20
S.D.	1.08	0.74	0.06
— Samples 596 + 603			
Mean		2.33	0.19
S.D.		0.41	0.03

Table 2. Time needed for different operations in treating 1 kg of fruits of *Parkia biglobosa.*

Sample No.	Shelling (min)	Pounding (min)	Sieving (min)	Washing in water (min)	Total (min)
596	48	4	4	5	61
598	28	4	2	3	37
599	25	4	3	5	37
600	31	4	3	4	42
601	26	4	3	4	37
602	29	3	2	3	37
603	28	4	3	4	39
604	29	4	2	4	39
605	37	3	3	2	45
607	30	3	3	2	38
610	32	3	3	3	41
611	33	2	1	3	39
612	31	5	3	4	43
613	30	5	3	3	41
614	29	3	3	2	37
615	32	3	2	3	40
616	27	3	3	3	36
Mean	30′ 53″	3′ 35″	2′ 42″	3′ 21″	40′ 31″
SD	5.09				5.658
−596					
Mean	29′ 49″				39′ 15″
SD	2.83				2.49

istic range — 12.15 ± 2.12 kg (10–14 kg) of fruits per man-day — was obtained.

4. Quantity of seeds per man-day After the treatment of fruits, pure seeds were left. The mean production of seeds per man-day for the samples was determined at 2.49 kg. (Because of the results in 3 above it was decided to omit sample 596 from further calculations).

The confidence interval at 95% level was determined at ± 1.46; so 2.49 ± 1.46 kg of seed are treated per man-day. It is now sample 603 that does not fit in the range. And when omitting sample 603 we find 2.3 ± 0.8. (1.5←2.3→3.1) kg of seeds per man-day.

5. Ratio seeds : fruits The production of seeds produced from 1 kg of fruits is given in Table 1. The mean ratio has been determined at 0.20. With respect to the S.D. and 95% level the range can be defined as 0.20 ± 0.12 (or : between 8 and 32). Again sample 603 does not fit in the range. When omitting 603 we find 0.19 ± 0.06. Those figures correspond to those of Busson (1965) who found 0.18.

6. Mean time needed for each phase of treatment of fruits From Table 2 it is clear that shelling takes about 75% of the time needed for treatment of fruit. The mean total time for the treatment of 1 kg of fruit has been calculated at 40′30″ (Table 2).

Considering the S.D. the range in time will be 40′30″ ± 11′5″. Treatment of sample 596 was extremely time consuming (see also 3 above) and does not fit in the range for the 95% level. Calculation of a more realistic range (omitting sample 596) gave: 34←39→45 for the treatment of 1 kg of fruit.

Discussion

The natural variation for the ratio seed : fruits is rather large. For example, for sample 603 (Table 1) almost 5 kg of seeds were obtained from 12 kg of fruits, while for sample 612 we did not even get 1.5 kg from about 11 kg of fruits.

In both cases the labourers needed the same time to treat about the same quantity of seeds, so it would not be fair to pay the labourers on basis of their net weight production of seeds. In our example the labourer for sample 603 would have earned over 3 times the salary as the one for sample 612, for basically the same effort.

Payment should therefore be calculated on the basis of kg of fruits treated. As indicated above, the mean daily production per man-day is 10–14 kg of fruits with an average of 12 kg.

Conclusion

Extraction techniques of *P. biglobosa* seeds and specifications for the optimal output of this processing were established. The mechanisation of processing is possible but operations such as shelling and sorting would be difficult to mechanise.

An immediate use of these results is to set piecework rates rather than hourly rates of payment for seed extraction of *P. biglobosa*.

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Effects of Temperature and Seed Moisture Content on the Storage of *Dendrocalamus brandisii* Seeds

P. Boonarutee and K. Somboon*

Abstract

Seed storage studies of *Dendrocalamus brandisii* Kurz were carried out with the objective to determine the effects of storage temperature and seed moisture content on the optimum duration of storage. It was found that *D. brandisii* seeds can be stored for 18 months under cold room temperature (2–4°C) without any reduction in the germination percentage. The effect of seed moisture content on storage life is small in this experiment. It might be possible to store *D. brandisii* seeds for a longer period than 18 months at low temperatures.

In Thailand, *Dendrocalamus brandisii* Kurz is one of the most important of the 18 species in the genus *Dendrocalamus*. Its vernacular names are Pai Bong Yai (Kanchanaburi), Sang Yen or Poa in Chiangmai. It is the thornless clump bamboo type of medium to rather large culm. In Thailand, the distribution of this species is in the western and northern parts of the country at an altitude of 100–1300 m. It is a native species of Burma, Thailand, Laos, and Vietnam.

In normal practice bamboo is propagated by culm cutting or clump or rhizome division rather than by seeds. This is because of nonregular supply of seeds due to unpredictable seed years and rapid loss of viability under normal room temperature storage conditions. Flowering and fruiting of bamboo normally occur in groups of rather large area; therefore abundant quantities of seeds can be collected in some seed years. In order to overcome the problem of irregular seed supply, techniques for prolonging seed viability have to be studied.

Studies have been conducted to determine the conditions for long-term storage of *D. brandisii* seeds to ensure future seed supply.

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Materials and Methods

D. brandisii seeds were collected from Kruang Kra Via Wildlife Sanctuary in Kanchanaburi Province in February 1987 and the storage experiment was initiated in March 1987, approximately a month after collection. The storage conditions in this experiment are as follows:

1) Control: seeds were stored at room temperature between 23 and 40°C.

2) Cold room storage: seeds were stored in the cold room at temperatures between 2 and 4°C.

For each set of storage conditions, there were two levels of seed moisture content as follows:

1) Seeds held at 11.75% moisture content. This level of seed moisture content had been reached by the normal practice of air drying seeds after collection and storing at normal room temperature until the experiment is initiated.

2) Seeds held at 5.23% moisture content. This level had been attained by oven drying the seeds at 40°C for 72 hours.

Seeds with the required moisture content were packed in two layers in small plastic bags in amounts sufficient for germination testing and moisture content monitoring at each storage period. Every three months after storing, seeds from all treatments were withdrawn for germination tests and moisture content assessment.

Seed moisture content was assessed for all seed treatments in the experiment. Twelve grams of

seed were taken from each seedlot for the germination test. The seeds were divided in to 4 lots of 3 grams each before being oven dried at 105 ± 2°C for 17 ± 2 hr, or until there was no weight reduction. The moisture content of seed was calculated by the following formula:

$$\text{per cent moisture content} = \frac{(\text{fresh weight} - \text{oven dry weight})}{\text{oven dry weight}} \times 100$$

The germination test for each treatment comprised 4 replications each of 100 seeds. Seeds were germinated in plastic box containers (11×11×8 cm) using vermiculite as a medium. Germination boxes were kept in the seed germinator at 30°C and with a light period of 8 hours. The number of germinants was counted every day for 20 days. Seeds that germinated until the radicle was longer than seed length were counted as good seeds. This storage experiment extended over 18 months.

Results and Discussion

This experiment showed that the percentage germination of *D. brandisii* seeds stored at room temperature (23–40°C) and at 2–4°C with seed moisture content 5.23% and 11.75% varied between 0 and 85 (Table 1). The effects of storage temperature and duration of storage on seed germination are significantly different ($P<0.05$). The effect of seed moisture content was not significant, and had less influence on seed germination post-storage in this experiment. The range of seed moisture content in this experiment may not have been wide enough to show its effects on seed viability. However, in this experiment the loss of seed viability seemed to be more rapid at the higher moisture content. At higher seed moisture content (11.75%), seed viability was totally lost within 8 months of storage compared to seed with the lower moisture content (5.23%) which lost its viability after 12 months (Table 1). At higher seed moisture content the trend of reduction in seed viability was more rapid even when the seeds were stored at low temperature. It was clear that loss of viability was related to the duration of storage.

Temperature is the major factor affecting seed viability in this experiment. Seed that were stored in the cold room (2-4°C) still retained high viability after 18 months, even at high seed moisture content. In this experiment there was no interaction between the effects of temperature and seed moisture content on seed germination as was reported by Ramyarangsi (1988) in *Thyrsostachys siamensis* seeds, another bamboo species. Bhumibhamon (1980) reported that the deterioration of seed quality depends on two environmental factors — relative humidity that regulates seed moisture content, and temperature — and that both influence the metabolic rate of seeds.

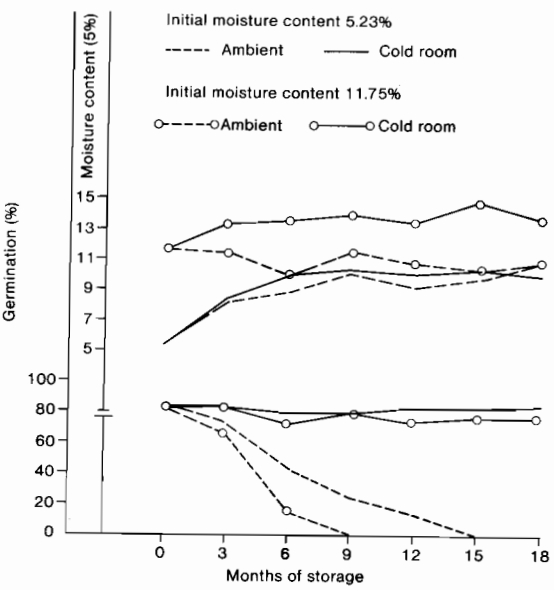

Fig. 1. Germination and moisture content of *D. brandisii* seeds for 4 treatments at 18 months storage.

Table 1. Three-monthly germination percentages and seed moisture contents of *D. brandisii* seeds under four conditions of storage.

Duration of storage (months)	Initial MC = 11.75%				Initial MC = 5.23%			
	Ambient		Cold room		Ambient		Cold room	
	MC	G	MC	G	MC	G	MC	G
0	11.75	83	11.75	83	5.23	85	5.23	85
3	11.4	66.25	13.32	82.25	8.01	72.5	8.31	82.5
6	9.97	15.5	13.42	71	8.72	43.6	9.8	78
9	11.42	—	13.74	78.75	9.89	25.75	10.29	78
12	10.69	—	13.23	72.5	9.09	13.75	9.03	81.25
15	10.19	—	14.64	75.75	9.73	—	10.17	80
18	10.68	—	13.52	75	10.73	—	9.74	82

Baldwin (1955) concluded that seed moisture content is the important factor affecting seed viability, and changes in seed moisture content are more detrimental to seeds than temperature changes. He also showed that a 1% reduction in seed moisture content can double the storage life of seed. In this experiment, the moisture content of seed during storage changed (Fig. 1). In sealed storage containers the moisture content of seed is the factor that determines the relative humidity in the container, because of the absorption and release of moisture from seeds to balance the surrounding atmosphere (Duangpatra 1986). The rate of absorption and release varies according to relative humidity and temperature. Besides this the moisture content of seeds already in balance with the surrounding atmosphere can also increase when temperature is reduced or when seeds deteriorate.

Conclusion

The studies on the storage of *Dendrocalamus brandisii* Kurz seeds under normal room temperature (23–40°C) and cold room temperature (2–4°C) with two levels of seed moisture content for 18 months has revealed that seeds stored in a cold room retain their viability longer than seeds stored at room temperature. There are interaction effects between temperature and duration of storage on seed viability. Even though seed moisture content did not affect seed viability significantly in this experiment, there is an indication that the seeds with lower moisture content retained their viability longer than those with a higher moisture content. Therefore seed moisture content and temperature can influence duration of seed storage and should be the major factors in the future studies of seed storage.

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Storage of Recalcitrant Seeds: Past, Present and Future

H.F. Chin*

Abstract

The moist or imbibed method of seed storage has been used over the past fifty years. Perennial crop seeds such as rubber (*Hevea brasiliensis*) have been successfully stored in this manner for about three months. It is a short term measure, unsuitable for long term conservation of genetic resources. The introduction of the term 'recalcitrant seeds' in 1973 increased awareness of this particular class of seeds and stimulated interest among seed technologists. This paper traces the history of storage of recalcitrant seeds, and describes and discusses the present storage techniques. Emphasis is given particularly to the new technique of cryopreservation of excised embryos of recalcitrant seeds.

THE general practice of seed storage in the past was based on the premise that all seeds must be dried to a low moisture content for storage. This statement occurred frequently in text books. Harrington's (1972) rule-of-thumb for seed storage stated that for every increase of 1% moisture content, the storage life is halved. However, it is now known this does not apply to all types of seeds. Roberts (1973) first introduced the terms 'orthodox' and 'recalcitrant' to describe the storage behaviour and requirements of seeds. For example, orthodox seeds like rice or wheat can tolerate desiccation to moisture levels below 5% and also tolerate freezing temperatures, while recalcitrant seeds such as rubber and cocoa are very sensitive to desiccation. They are killed if their moisture content is reduced below a relatively high critical value, within the range of 12–31%.

Recalcitrant seeds do not tolerate freezing temperatures and hence they are difficult to store. Under moist conditions they will maintain their viability for only a few weeks, usually less than 12 weeks. Hanson (1984) was of the opinion that the term recalcitrant seeds will be more meaningful and easily understood if they are referred to as desiccation-sensitive and orthodox seeds as non-desiccation sensitive. More recently, Pammenter et al. (1989) proposed the term 'homoiohydrous' in place of 'recalcitrant' as the development of these seeds on the parent plant does not culminate in the maturation drying phase and once shed, they exhibit desiccation sensitivity. Whatever name we call them, this group of seeds is desiccation-sensitive and presents many storage problems. To date there is no method available for their long-term storage (Chin 1988).

Recalcitrant seeds form a very important group in the seed industry of major plantation crops, especially many important tropical timber species. Little attention has been focused on the storage of the tropical fruit species (Hanson 1984), and Chin (1978) has stressed there are many problems yet to be solved for the tropical plantation crop seeds industry. Williams (1984) recommended seed research on major clonal crops should be greatly accelerated. It was pleasing to note at the 22nd International Seed Testing Association (ISTA) Congress held in Edinburgh in June 1989 that more than 60% of papers on seed storage concerned recalcitrant seeds.

Research activities and publications on recalcitrant seeds have increased since 1973. King and Roberts (1979) produced a comprehensive and useful literature review on recalcitrant seed for the International Board for Plant Genetic Resources (IBPGR) and subsequently a book on recalcitrant

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crop seeds was published (Chin and Roberts 1980).

This paper contains a status report on this subject, highlighting the characteristics of recalcitrant seeds. It covers techniques used in the past and present, and in particular presents the latest development of a new technique of cryopreservation of excised embryos of recalcitrant seeds. This technique may be a new alternative to storage of recalcitrant seeds in the near future.

Characteristics of Recalcitrant Seeds

Recalcitrant seeds have been classified according to their behaviour, i.e. their sensitivity to desiccation and intolerance to freezing temperatures. In order to understand and know these seeds better it is essential to establish some of the more common general characteristics, noting the exceptions. A good knowledge of their characteristics and properties will help to solve their storage problems.

Recalcitrant seeds are generally very large in comparison to orthodox seeds. They exist under a moist ecological forest-type environment. They are also very heavy, with 1000-seed weight often exceeding 500 g (Chin et al. 1984). This is also attributed to their high moisture content (ranging from 30 to 70%) at time of shedding from the parent plant. There is also a great deal of variation in moisture content between individual seeds with a high coefficient of variation of about 7–13% compared to 2 or 3% in orthodox seeds.

Seeds belonging to the recalcitrant group are very sensitive to desiccation but they differ in degree of sensitivity. For example, the critical moisture content levels which kill seeds are: *Shorea talura* 17% (Sasaki 1976); *Hevea brasiliensis* 15–20% (Chin et al. 1981); *Nephelium lappaceum* 20% (Chin 1975); *Theobroma cacao* 26% (Hor et al. 1984); *Hopea helferi* 35% (Tamari 1976). Recalcitrant seeds are also sensitive to low freezing temperatures. Seeds of many tropical species are even killed at subambient temperatures or suffer from chilling injury. Examples are: *Theobroma cacao* (Hor et al. 1984); *Nephelium lappaceum* (Chin 1975); *Dryobalanops aromatica* (Jensen 1971); *Hopea odorata* (Tang and Tamari 1973); *Shorea ovalis* (Sasaki 1976); *Garcinia mangostana* (Winters 1953). Hor (1984) showed there was a very sharp reduction in storability of cocoa seeds at 15°C compared to 17°C, indicating that they are very sensitive to slight temperature reduction around a critical value. The temperature difference of 2°C means that seeds die in less than 2 weeks and suggests that only a few interrelated reactions may be involved in causing death.

A survey of the moisture content of individual seed and parts of seed showed considerable variation between seeds and their parts (Chin et al. 1989b). The variation in moisture content of individual seeds of orthodox and recalcitrant species does not vary significantly with coefficient of variation of 2.8% in the former compared to 7% in the latter. All the variation between individual seeds can be attributed to the often irregular results of experiments on the storage of recalcitrant seeds. Finally, Chin et al. (1987) showed excised embryos of recalcitrant species are more tolerant to desiccation than whole seeds. Thus it is possible to cryopreserve excised embryos of recalcitrant seeds.

Past and Present Methods of Storage

Moist or imbibed storage techniques have been in use for a long time. Some are still practical for short-term storage. There are many variations of moist or imbibed techniques ranging from storage between moistened papers to the extreme of soaking the seeds in water. The moist method is still the best, and for many years rubber seeds have been stored in between moist sawdust or charcoal at 7°C. The storage life is 2–3 months. King and Roberts (1979) listed all the various storage media and periods of storage for many temperate and tropical species.

Many other storage methods have been attempted but all have failed. For example inhibitors have been used to prevent sprouting during storage of cocoa (Pyke et al. 1934) and rambutan (Chin 1975). Storage in airtight containers has been tried with durian (Soepadmo and Eow 1976). Other methods include *Shorea talura* sealed in polythene bags (Sasaki 1976), cocoa sealed in an atmosphere of CO_2 (Villa 1962), and rubber seeds in brine and polyethylene glycol (Sakhibun 1981). All these methods have met with little success: as the seeds have remained viable for only a few weeks. The most extreme form of imbibed storage is to store seeds in water. Ong and Lauw (1963) stored rubber seeds under water and after one month the percentage germination was above 50%. King and Roberts (1982) also stored cocoa seeds under water using the imbibed storage technique.

In the last decade a partial desiccation technique has been developed for cocoa and rubber. This method involves partial drying the seeds with cool air (20°C). Freshly harvested rubber seeds at above 20% moisture content were cleaned, surface dried and treated with a fungicide, 0.3% benlate (Normah et al. 1986). Hor (1984) stored cocoa seeds, partially dried and dressed with benlate, in lots of 500 in perforated

plastic bags. Storage of cocoa seeds improved from 3 to 6 months, and rubber seeds to over a year. The partial desiccation and moist storage methods are still in use.

Future Methods of Storage

The outlook for long-term storage of recalcitrant seeds as genetic resources is bleak. Over the years many attempts have been made, but little progress has been achieved. To date there has been no success in storing truly recalcitrant seeds over a long period, as the technical problems are formidable. According to Roberts et al. (1984) the most promising method of germplasm conservation for recalcitrant species is storage in liquid nitrogen. This may be unexpected for seeds that are very sensitive to freezing temperatures. Withers (1980) and Bajaj (1985) suggested that in cases where seeds are short-lived, germplasm could possibly be conserved through cryopreservation of excised embryos or their segments. Grout et al. (1983) have shown that embryos of oil palm (*Elaeis guineensis*) seeds, which were formerly classified as recalcitrant, can be successfully cryopreserved after desiccation. Grout (1979) has used low temperature storage of imbibed tomato seeds as a model for recalcitrant seed storage. With this supporting evidence it is now clear that cryogenic storage is a strong possibility for the conservation of genetic resources of recalcitrant species.

Over the last five years, research in Universiti Pertanian Malaysia has been directed towards cryopreservation of excised embryos of recalcitrant species. In the initial attempt with rubber seeds, excised embryos survived liquid nitrogen but the percentage survival was not high (Normah et al. 1986). Successful recovery of viable excised embryos from liquid nitrogen and their subsequent regrowth in vitro shows this method can be further developed into a practical technique for long-term conservation. The discovery by Chin et al. (1987) that excised embryos of recalcitrant seeds of jackfruit (*Artocarpus heterophyllus*) can tolerate desiccation down to 10% paves the way for further rapid development of this technique. It has been known for a long time that seeds must have a low moisture content to survive in storage at very low temperatures (Lipman and Lewis 1934; Sakai and Nishiro 1975). Whole seeds of recalcitrant species cannot be desiccated to such low moisture and hence can never be cryopreserved.

Since the discovery that excised embryos have reduced sensitivity to desiccation, some progress has been achieved in the cryopreservation of excised embryos of jackfruit seeds, with a survival rate in excess of 60%. This will serve as a model, and the technique is being revised and refined. Chin et al. (1989a) devised an optimum protocol for successful storage of these embryos in liquid nitrogen. Recalcitrant seeds are well known for their variability in size, maturity, structure and moisture content (Chin et al. 1989b), but embryos of more even size, measuring 4–5 mm show little variation. Embryos have a critical moisture content of 14–15%, below which abnormality in seedlings is encountered.

Cryoprotectants are required for successful cryopreservation. Desiccation alone is not enough. In the case of jackfruit, embryos were protected with 10% dimethyl sulphoxide and 0.5% proline solution for 12 hours. They were blotted dry and desiccated for one hour, then cooled slowly at approximately 1°C per minute down to −40°C before preserving in liquid nitrogen at −196°C. Survival rate after in vitro culture was around 60%. The embryos recovered from cryostorage were generally slow to develop in the medium. However, a medium enriched with gibberellin and amino acids enhanced seedling growth (Chin et al. 1988). Seedlings were transplanted into polybags and kept under mist in a propagator to minimise transplanting shock. Well developed, normal cryopreserved seedlings were obtained, but their growth rate is slower and initial leaves are also smaller. The growth and development of these plants will be continuously observed. This successful technique when further refined, is a potential method for the conservation of recalcitrant species. With the rapid development and advancements in tissue culture and biotechnology, I am confident that in the near future cryopreservation of excised embryos will become a practical method for conservation of recalcitrant species.

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Predicting the Storage Life of Orthodox Tropical Forest Tree Seeds

P. B. Tompsett*

Abstract

An equation developed by E.H. Roberts and R.H. Ellis from seed storage experiments on 'orthodox'-seeded agricultural species has been applied to tropical forest tree seeds. The equation includes five constants: two temperature constants, C_H and C_Q, each of which is believed to have a common value applicable to seeds of all species; two species constants, K_E and C_W, which can now be estimated relatively quickly; and K_i, the seedlot constant, which can be assessed as the intercept of a seed survival curve at zero time. These constants can be used to predict storage life for any homogeneous seedlot over a wide range of storage conditions.

AN 'ORTHODOX' seed can be dried to a low moisture content without loss of viability. The longevity of such seed can be predicted by determination of the constants in a viability equation which was developed at Reading University (Ellis and Roberts 1980a, 1980b; Ellis 1984, 1988). The equation has been applied to tropical forest tree seeds (Tompsett 1986) and a quick and relatively easy method for determination of the constants has been proposed (Ellis et al. 1986; Dickie et al. 1989).

Basis for the Viability Equation

Seed Storage Life

It is impossible to determine the storage life of an individual seed because of the destructive nature of a viability test; the seed either dies before the test or else germinates so that the time of its death cannot be established. We thus have to assess the viability of a seedlot from a sample. If successive samples of a seedlot are taken during storage under constant conditions a particular pattern of germination percentage against time is observed. In the illustration for *Swietenia humilis* in Fig. 1 it can be seen that the decline in the observed points approximates to a sigmoid pattern, referred to as the survival curve. One measure of a seedlot's longevity is the period of time for seed to decline to 50% germination (p_{50}). It is worth noting that the p_{50} value will be less if the seedlot is stored at a lower initial percentage germination.

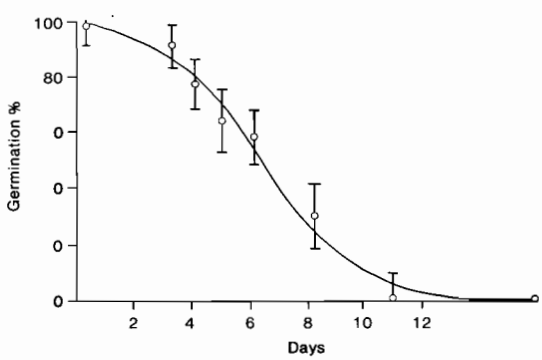

Fig. 1. The results and standard deviations of germinations on eight samples of *Swietenia humilis* seed stored over a period of 16 days at 62°C and 3% moisture content. The solid line is the survival curve, fitted to the data by probit analysis.

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Rate of Loss of Viability and Seed-to-seed Variation in Storage Life

The sigmoidal curve shows that individual seeds become ungerminable at different times during storage; a steep slope shows there is less seed-to-seed variation than a shallow slope. The mean rate of loss of viability is the reciprocal of the mean longevity of all seeds in the population.

To understand the basis for the viability equation, it is helpful to consider the frequency distribution of seed deaths over time in storage. Provided conditions are constant, a bell-shaped curve is produced, indicating that (per unit of time and for a seedlot starting near 100% germination) fewer deaths occur at the start and at the end of storage than at the p_{50} point (see the lower line of Fig. 2); there is a normal distribution of seed deaths over time. By replotting the data as percentage germination, we can again produce the sigmoid shape of curve referred to earlier; this curve is the corresponding negative cumulative normal distribution (upper line in Fig. 2). The frequency distribution can thus be described by the parameters p_{50} and standard deviation σ, as shown in Fig. 2.

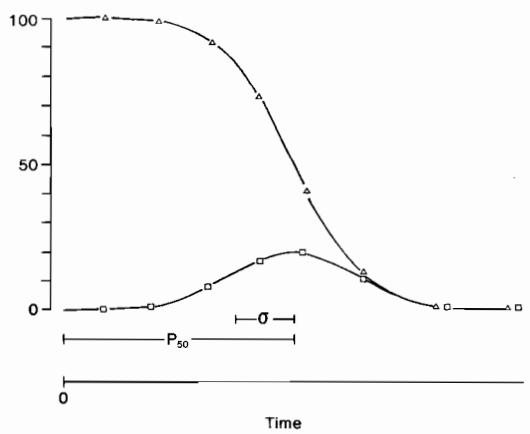

Fig. 2. The lower curve is a normal frequency distribution of seed deaths per unit time; the upper curve represents the same data plotted as percentage germination. The scales are (i) germination plotted against time and (ii) the number of seeds that have died in the interval since the previous sample, plotted against time. The p_{50} is the time for the seed batch to reach 50% germination and the σ is the standard deviation of the distribution of seed deaths in time. Adapted from Ellis (1984).

Use of Probability Scales

The sigmoid seed survival curves can be replotted on probability graph paper to give a straight line (Fig. 3); random variation due to sampling error can be observed around the line. The

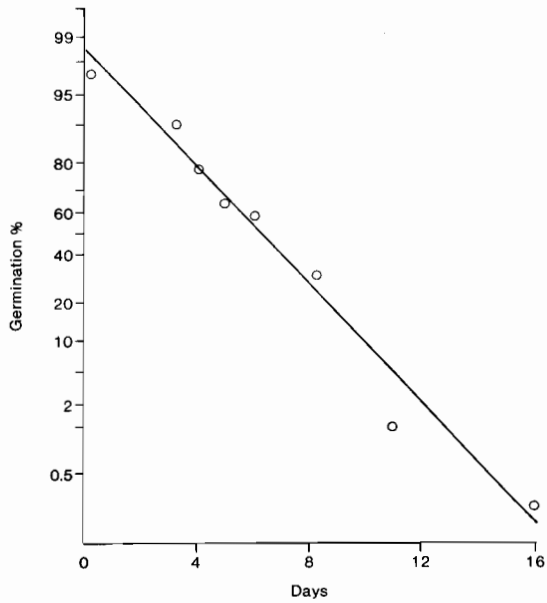

Fig. 3. The data from Fig. 1 are replotted here on probit graph paper; this transformation converts the sigmoid curve to a straight line. Sampling error accounts for deviation of the points from the line.

straight line plot can also be determined if percentage germination is firstly transformed to probit values, as is shown in Fig. 4. A probit value of 1 is

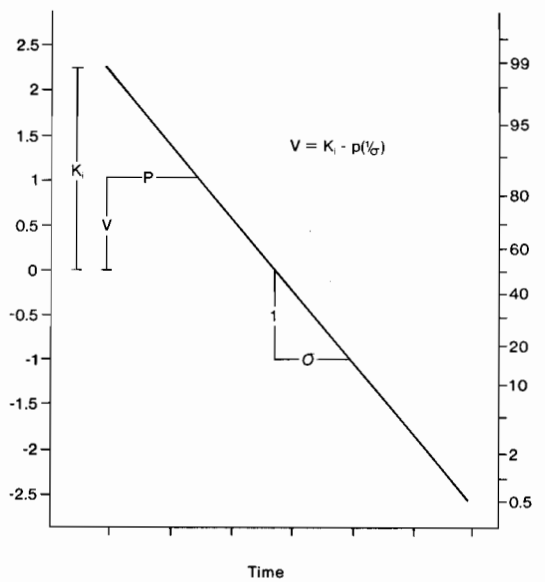

Fig. 4. Diagram to illustrate the seed survival curve when percentage viability was transformed to probit percentage viability (V). For comparison, percentage germination is presented on a probability scale on the right hand side. From Ellis (1984).

one standard deviation above the mean; as illustrated in Fig. 4, the slope of the line of probit germination against time is the reciprocal of standard deviation (i.e. $1/\sigma$). Thus, the equation of the line is:

$$V = K_i - p\ (1/\sigma) \qquad (1)$$

where p is storage period (days), K_i is the origin of the line at day zero and σ is the standard deviation of the distribution of seed deaths over time. It is apparent that σ, like p_{50}, is a measure of the longevity of the seedlot; it increases in direct proportion as longevity is increased.

An efficient method to determine the parameters of the line is by linear regression, weighting in favour of data close to the p_{50} point on the horizontal scale; this can be done by probit analysis.

Influence of Storage Environment

Both increased moisture content and increased temperature reduce storage life. This pattern can be observed in data for the tropical tree Terb (*Terminalia brassii*) (Fig. 5); longevity is presented as $\log_{10}\sigma$ for reasons described below. It has been found, however, that above a critical moisture content (15–28%, depending on species) the trend for reduced longevity with increased moisture content is reversed. Likewise, there is a lower limit, below which the relationship no longer holds (generally below 3–5%).

Certain tropical trees with 'orthodox' seed physiology may lose some viability irretrievably on drying to 7% moisture content; examples of this type are *Araucaria columnaris* and *Dipterocarpus alatus* seeds. The latter problem can sometimes be alleviated by holding seeds at high relative humidity before sowing; in other cases, a period of soaking before sowing has been beneficial. This desiccation effect need not, however, affect the expected relationship between moisture content and longevity (Tompsett 1984). A recent review of water and seed survival is that of Roberts and Ellis (1989).

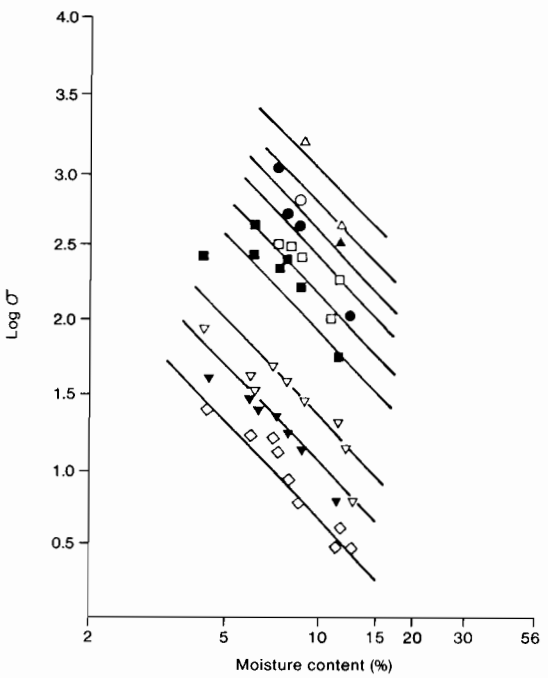

Fig. 5. The influence of storage environment on the longevity of *Terminalia brassii* seed. Solid lines show the predicted relation between longevity (log σ) and moisture content, determined after fitting Eqn (5). Temperatures used were 42°C (◇), 36° (▽), 31°C (▼), 21°C (□), 16°C (■), 11°C (●), 6°C (○) 2°C (▲) and −4°C (△) (Tompsett 1986).

Differences between Populations of Seeds within a Species

Differences in storage life between seedlots can occur despite storage under identical conditions. Such differences can be because of differences in the K_i values of the seedlots; these differences are, however, not always obvious from initial germination tests (such tests give a less accurate estimate of initial viability than a K_i value from a well-defined survival curve). Examples are presented in Fig. 6; it should be noted that the seedlot

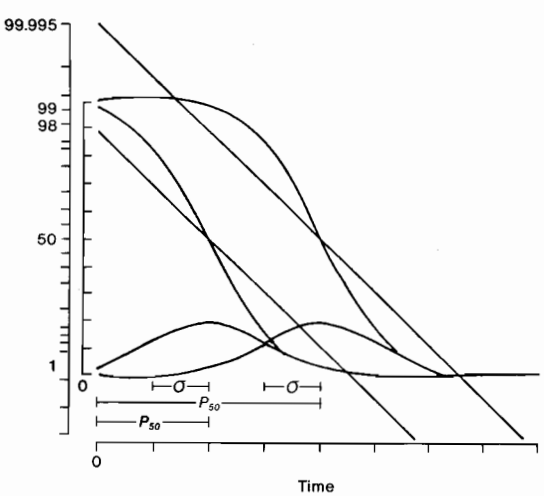

Fig. 6. Diagram to illustrate survival curves of two seedlots stored in identical conditions and at similar initial germination percentages (but, as the difference in initial probit percentage germination is 1.2, a different degree of aging has been experienced by the seedlots before storage). The survival curves are identical but displaced from one another. From Ellis (1984).

with the lowest initial viability (K_i) will survive for the shortest time but that the mean rate of loss of viability (which is proportional to $1/\sigma$) is the same for both seedlots.

A second way in which seedlots can differ in their longevity is by being in different states of dormancy. It is known for rice, however, that six different cultivars gave the same survival curves after completion of various periods of after-ripening to remove dormancy (Roberts 1963). Thus, the germination rose initially during storage but, after passing a peak, a sigmoid curve of the same shape was produced in all six cultivars. It is important, therefore, that dormancy should be removed before viability studies are made.

Factors other than Environment which may Affect Standard Deviation

Two factors may lead to incorrect estimation of σ. Firstly, it is essential that the seedlot in question is homogeneous. If two homogeneous seedlots, each with its own K_i, are mixed together equally, an intermediate survival curve is produced; such a curve is illustrated in Fig. 7.

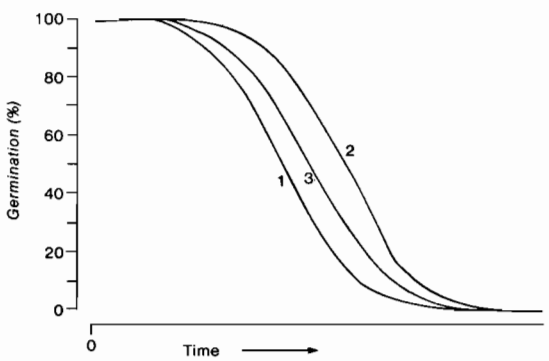

Fig. 7. Seed survival curves of two seedlots stored in the same conditions (curves 1 and 2) with an intermediate curve (3) to show the result using a mixture of the seeds from curves 1 and 2. From Ellis (1984).

A second potentially confounding factor is the presence of seeds in the seedlot which fail to germinate for reasons other than loss of viability through normal aging. Three such categories are often found in tree seedlots: empty seeds; insect infested seeds; and seeds which have blackened but solid embryos (e.g. in *Dipterocarpus*). The number of such seeds should be carefully assessed and deducted from the number of seeds sown before any analysis is performed.

Quantitative Effect of Temperature on Seed Longevity

One way to examine the relationship between seed longevity and temperature is to plot relevant data and examine the curve produced. If the standard deviation σ is plotted on a log scale against temperature a straight line is produced, at least over a narrow range of temperatures around 20–30°C. Thus,

$$\log_{10}\sigma = K - Ct \quad (2)$$

where K is the intercept, C is the gradient and t is the temperature (°C).

It is necessary, however, to know how longevity changes over a much wider range of temperatures. For this purpose, it is useful to examine the effect of temperature on Q_{10} (the ratio of the rate of loss of viability at one temperature to the rate at a temperature 10°C lower). Such plots have clearly shown that the relationship is curvilinear rather than linear; the predicted relationship for *Swietenia humilis* calculated from a substantial data set is given in Fig. 8 and many other species give consistent results. A quadratic relationship is thus appropriate:

$$\log_{10}\sigma = K - C_H t - C_Q t^2 \quad (3)$$

where C_H and C_Q are temperature constants.

The pooled experimental data from three laboratories have supplied evidence that C_H and C_Q do not differ between species; the respective

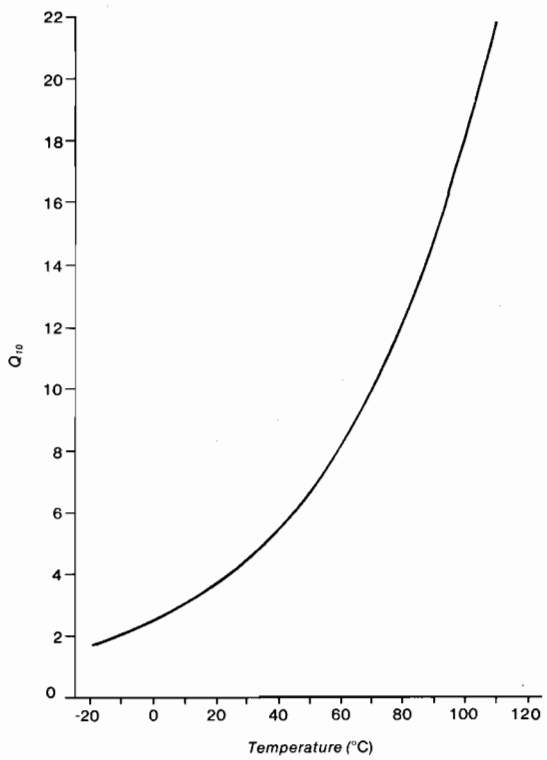

Fig. 8. The relationship between the temperature coefficient for the rate of loss of viability, Q_{10}, and temperature for *Swietenia humilis* seed.

values 0.033 and 0.000478 were determined from substantial experiments on eight widely different species (Dickie et al. 1989).

Quantitative Effect of Seed Moisture Content on Longevity

An asymptotic relationship between $\log_{10}\sigma$ and moisture content is suggested by experimental data and the following equation is found to fit well:

$$\log_{10}\sigma = K - C_w \log_{10} m \quad (4)$$

where K is an intercept constant at 1% moisture content, m is the moisture content (% f. wt) and C_W is a constant for the gradient. Unlike C_H and C_Q, the C_W constant varies considerably among species, being high in non-oily seeds (e.g. barley at 5.9) and low in oily seeds (e.g. *Terminalia brassii* at 3.0).

Combined Effect of Moisture Content and Temperature on Longevity

Combining equations (2) and (4) produces

$$\log_{10}\sigma = K_E - C_W \log_{10} m - C_H t - C_Q t^2 \quad (5)$$

where K_E is a constant representing the intercept on the longevity axis of a three-dimensional graph of longevity against temperature against moisture content, at the point where temperature is 0°C and moisture content is 1%. The four constants do not differ among seedlots of the same species (Ellis et al. 1982).

A single viability equation can be produced by combining equations (1) and (5):

$$V = K_i - p/10^{K_E - C_W \log_{10} m - C_H t - C_Q t^2} \quad (6)$$

Application of the Viability Equation

Estimation of the Five Constants

The seedlot constant, K_i, may be estimated by a rapid aging test in which a survival curve is determined quickly at a convenient combination of moisture contents and temperature; K_i is the intercept at time zero on the vertical axis.

Eight species (barley, cowpea, chickpea, soybean, lettuce and the forest trees *Terminalia brassii*, elm and mahogany species) on which the most accurate assessments of the constants have been made were examined over wide ranges of moisture content and temperature. Constant moisture contents were assured by hermetic sealing. Samples were taken from each storage environment for viability determination at intervals to enable construction of transformed survival curves for which σ values were calculated. The results from one such experiment on *T. brassii* seed are presented in Fig. 5; multiple regression analysis on the basis of equation (5) produced values for K_E, C_W, C_H and C_Q. The parallel lines in Fig. 5 represent the longevities predicted by the viability constant values presented for *T. brassii* in Table 1.

Quick Estimate of Viability Constants

To obtain results such as those in Fig. 5, much work is required over a considerable period of time. Since the temperature constants C_H and C_Q are not significantly different among eight species studied in depth (see above), a short-cut method, based on one originally proposed for sesame seed (Ellis et al. 1986), can now be proposed (Dickie et al. 1989). This method involves estimation of the survival curves for seed kept at several different moisture contents but at a single convenient temperature. The σ values thus acquired can then be used to determine the constants K_X and C_W by linear regression using the following equation, which is a simplification of Eqn (5) for the case where a single temperature is used:

$$\log_{10}\sigma = K_X - C_w \log m \quad (7)$$

Using the value of K_X obtained, K_E can be calculated from the equation

$$K_X = K_E - C_H t - C_Q t^2 \quad (8)$$

The values for C_H and C_Q from pooled data for eight species may be substituted in this equation ($C_H = 0.0329$; $C_Q = 0.000478$) (Dickie et al. 1989) in order to estimate K_E.

Table 1. Viability constants determined for tropical forest tree seeds. Values in parentheses are those obtained when K_E and C_W are constrained to the well-determined C_H and C_Q values.

Species	K_E	C_W	C_H	C_Q	Source
Terminalia brassii	5.00 (5.02)	2.15 (2.16)	0.035 (0.033)	0.000410 (0.000478)	Tompsett (1986)
Swietenia humilis	5.19 (5.39)	2.45 (2.39)	0.033 (0.033)	0.000499 (0.000478)	Tompsett (unpublished)
Araucaria columnaris	(4.68)	(1.86)	(0.033)	(0.000478)	Re-worked from Tompsett (1984)
*Dipterocarpus alatus**	(4.82)	(1.60)	(0.033)	(0.000478)	Tompsett (unpublished)
*Entandrophragma angolense**	(4.15)	(1.66)	(0.033)	(0.000478)	Tompsett (unpublished)

*Preliminary data

The values of K_E and C_W thus determined can then be used for prediction of longevity using Eqn (7) in conjunction with the pooled estimates of C_H and C_Q. The ranges over which such predictions can be made extend generally over temperatures from −20 to 90°C and over moisture contents in the range 5–15% for oily species and 5–25% in non-oily species.

Well determined constants for *Terminalia brassii, Swietenia humilis* and *Araucaria columnaris* and preliminary constants for *Dipterocarpus alatus* and *Entandrophragma angolense* are presented in Table 1; experiments on the latter two species are still under way. Values in parentheses were calculated by constraining K_E and C_W to the accurately-determined C_H and C_Q values; the resulting sets of constants are thus based on a much larger body of data and are consequently more accurate, assuming the temperature constant values are universally applicable.

Use of the constants may be illustrated by calculating the storage life of *Terminalia brassii* seed (using constants from Table 1) and barley seed (constants from Ellis and Roberts 1980b) stored at 5% moisture content and 2°C. If seeds are initially at 90% germination, they decline to 50% in 2114 and 9 years respectively, according to the predictions of equation (6). This illustration shows the typically large difference in seed longevity between tropical tree and grain species. The constants may also be used to determine the best methods for drying seeds and the order in which different seedlots of a species should be removed for plantation.

Acknowledgments

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IDS-Sedimentation of *Cupressus lusitanica* Seeds

U. Bergsten and M. Sundberg*

Abstract

Seeds of *Cupressus lusitanica* with low germination percentage (about 20%) were conditioned in two experiments using the following treatments:

Exp. 1. A. Cold-wet treatment at 5°C for 3 weeks.
B. Drying in dehumidified air until a near maximum difference in density between dead and viable seeds was obtained.
C. Separation in a sedimentation flume to achieve a gradient of fractions.

Exp. 2. The seeds were initially incubated at 15°C for 3 days and then subjected to the same treatments as in Exp. 1 but in the order B + C + A.

In both experiments the germination percentage of the best fractions was improved to about 60%. It was also found possible to get a good estimate of germination percentage using x-ray analysis if only filled seeds with discernible embryos were counted as germinable.

CUPRESSUS lusitanica is an exotic softwood species in East Africa which is commercially important in countries such as Ethiopia, Kenya and Tanzania (cf. Tadele 1988; Rode 1988; Shehaghilo 1988).

The interest in the above species has lead to research on growth and yield (Mathu and Philip 1979; Örlander 1986) and wood characteristics (e.g. Lema et al. 1978; White et al. 1980). Although there is a demand for this species it seems to be difficult to achieve high seedling emergence in nurseries (Tadele, pers. comm.) and, consequently, to produce enough seedlings. One reason for low seedling emergence could be the poor seed quality of this species (cf. Shehaghilo 1988).

For some conifer species, the use of new methods to eliminate non-productive seeds (for reviews, see Simak et al. 1985) has considerably improved seed quality. In the present work, one of these, i.e. IDS method, was tested on a poor quality seed lot of *Cupressus lusitanica* from Kenya. The IDS method is based on the principle that viable seeds after incubation (I) and subsequent drying (D) lose absorbed water at a much slower rate than dead seeds during a certain period of drying, in which the two types of seeds owing to the different density can be separated (S), for instance by flotation (Simak 1981) or sedimentation (Bergsten 1988) in water.

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Materials and Methods

The seed sample used was provided by Kenya via the Agricultural Research Institute through Danida Forest Seed Centre. Seeds were collected in 1982 from Daraja 2 (G) seed stand (2377 m.a.s.l.). The germination percentage of the seed lot was only about 20% (Fig. 1). The seed lot was subjected to the following treatments.

Experiment 1

A. Cold-wet treatment (syn. moist prechilling). Such treatments are recommended for some *Cupressus* species (ISTA, 1985). No specific recommendation is made for *C. lusitanica*. This treatment was used both for dormancy breaking and as the I-step in the IDS method (Simak 1981).

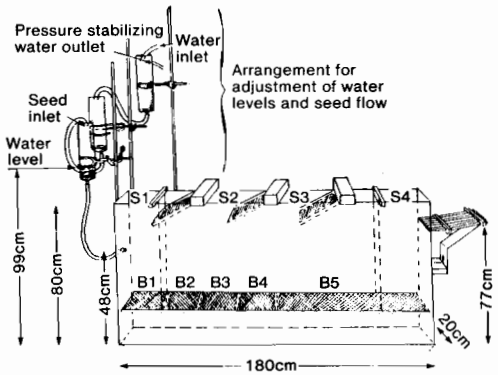

Fig. 1. The sedimentation flume showing how fractions B1–10 and S1–3 were collected.

The seeds were soaked instantaneously (within one minute) in tap water, pH about 7, at about 10°C, placed on moist blotting paper in plastic boxes covered with perspex plates at 5°C for 3 weeks, and then dried.

B. Drying-step (D-step in the IDS method). The seeds were dried using a rotating (0.5 rev/min) net drum (Ú 20 × 60 cm). The drum was placed in a drying cabinet supplied with dehumidified air (15% RH) at 20–25°C. The drying was performed until a near maximum difference in density between viable and dead seeds was reached. This difference was estimated by using a sink test in water on samples of 50 seeds. When the percentage of floating seeds was almost the same as the amount of dead seeds estimated from the previous seed analysis, the seeds were separated.

C. Separation in a sedimentation flume (a variant of the S-step in the IDS method (Bergsten 1987; 1988). The flume (Fig. 1) enabling vertical and horizontal separation consisted of ten compartments for sunken seeds (B1-B10, each with a length of 18 cm) and three compartments for floating seeds (S1: 30 cm, S2: 40 cm, S3: 90 cm). The seeds of each compartment were collected separately. In the analyses the fractions B5-B10 were pooled as they contained few seeds. The flume was used because of the non-uniform density of the seed lot, i.e. tests revealed that simple flotation in water was not possible as some non-germinable seeds sink even in dry condition. Therefore a combined vertical and horizontal separation in water was considered to be more appropriate. After this separation the seeds of each fraction were subjected to seed analysis.

Experiment 2

In this experiment the IDS schedule was used before cold-wet treatment, i.e. the seeds were soaked instantaneously and then placed between wet blotting paper in an incubator (Inventum DK 11) at 15°C for 3 days. The seeds were then dried, separated and given a cold-wet treatment for 3 weeks at 5°C. Conditions were the same as in Exp. 1; it was just the order of treatment which was different.

Seed Analysis

Before the germination test, seeds were x-rayed (Simak 1980) in replicates of 100 seeds and classified as follows:

X-ray class 0 = empty seeds
X-ray class 1 = filled seeds, embryo not discernible
X-ray class 2 = filled seeds, embryo discernible

Radiographs were made in a Faxitron x-ray machine at 15 kV, 3mA, 1.3 min, focus 55 cm, using Agfa-Gevaert D7 film.

The germination analysis (3×100 seeds) was carried out on Jacobsen apparatus at constant light (Thorn T 40 W/33 cool light, 20 $\mu Em^{-2}s^{-1}$) at 20°C for 21 days. Seeds with radicle of at least the same length as the seed were regarded as having germinated.

Results and Discussion

In both experiments the germination percentage of the best B-fractions was improved to about 60% (Fig. 2). There was a clear trend in both experiments that the germination percentage decreased with decreasing density of the D-dried seeds. A possible reason for this effect is that in seed lots with non-uniform density the most poorly developed seeds have the lowest density. Furthermore, and most important, high water holding capacity during drying (cf. D-step) is related to high viability of the seed and vice versa (Simak 1981).

There was a good correlation between percentage of seeds in x-ray class 2 and germination percentage (Fig. 3). In fact, for this seed lot it was possible to get a very good estimate of germination percentage just by counting the seeds in x-ray class 2. Radiography should therefore be a valuable tool when analysing seeds of this species.

Experiment 2 starting with an initial incubation before separation and subsequent cold-wet treatment showed more distinct differences in germination percentage between fractions. However, due to lack of seed it was not possible to repeat the experiments, so the optimum sequence order can only be estimated. The results do, however, indicate that seed lots of *Cupressus lusitanica* can be successfully improved using the IDS method although exact procedures need to be worked out.

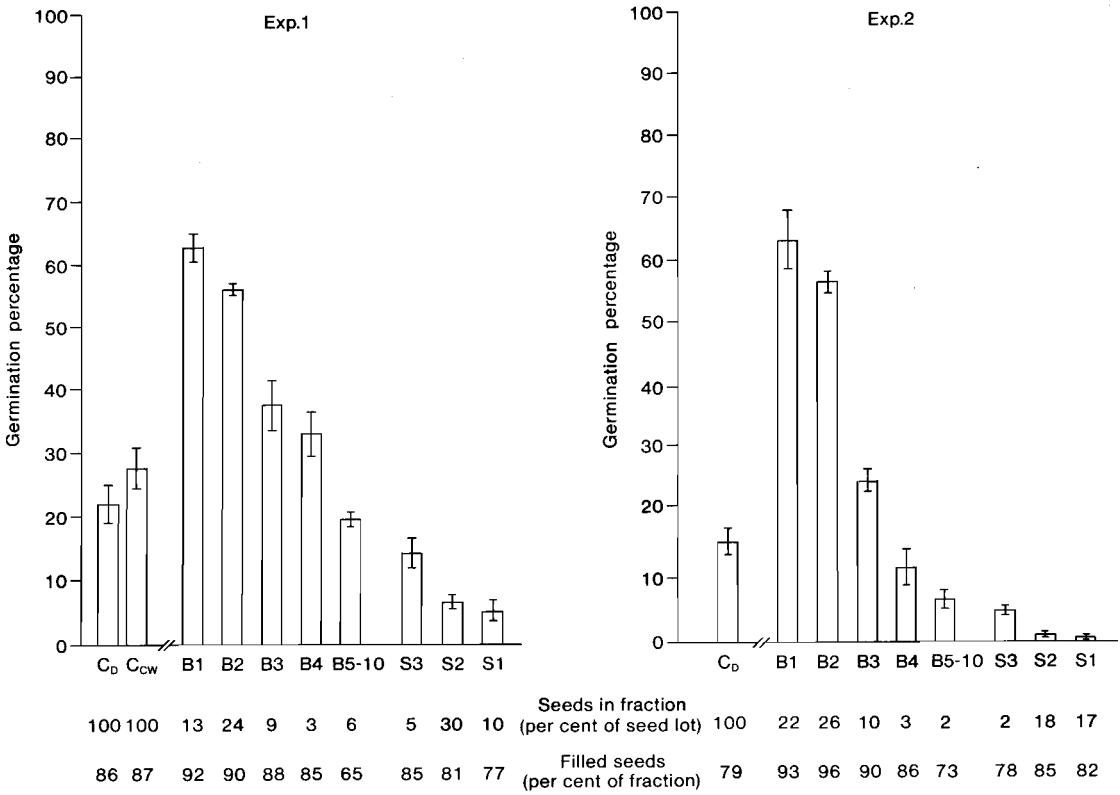

Fig. 2. Germination percentage for the B1–B10 and S3–S1 fractions after separation using the sedimentation flume. Vertical bars represent standard errors of the means (3 × 100 seeds) C_D = Control$_{dry}$ C_{CW} = Control$_{cold\text{-}wet}$

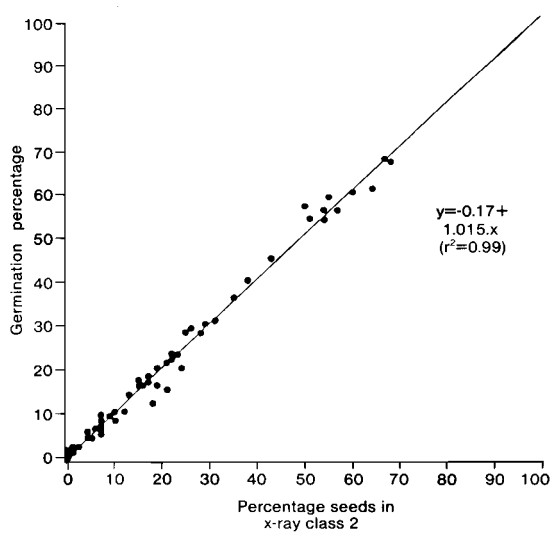

Fig. 3. The relationship between germination percentage and the percentage of seeds in x-ray class 2. Data are taken from all replications of all the treatments in both Exp. 1 and Exp. 2.

A possible regime might be to eliminate nonproductive seeds of the seed lot in a seed conditioning plant and leave the cold-wet treatment for the nursery.

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Investigation of Mycoflora and Pathology of Fungi Present on Stored Seeds of Australian Trees

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Abstract

Seed lots of *Acacia* spp., *Casuarina* spp., and *Eucalyptus* spp. stored at the Australian Tree Seed Centre (ATSC), Canberra, Australia were examined for the presence of fungal contamination. Twenty five fungal genera representing at least 38 species were recovered from the eighteen seed lots, and 27 isolations were new records from seed. Fungi observed in this study are widely distributed geographically and pose little quarantine threat. However the diversity of species isolated from seed coats, chaff and leaf debris indicates that more dangerous pathogens could readily be disseminated on the seed of Australian native trees.

AUSTRALIA is guardian to a unique and extensive resource of woody flora which has proven to be very valuable for tree planting programs both in Australia and overseas. There are over 6 million hectares of *Eucalyptus* plantations in the world, about one million of *Acacia* species, half a million of *Casuarina* and many other plantings of *Hakea, Grevillea* and *Araucaria.* These are playing an increasingly important role in the industrial and economic development of many countries.

The Australian Tree Seed Centre (ATSC), part of CSIRO's Division of Forestry and Forest Products, has acted for 25 years as a national tree seed bank. It supplies seeds to researchers in Australia and over 100 other countries. It is a national focus for both the import and export of tree seed and a recognised source of knowledge of the practical use of the Australian flora (Midgley 1988). The seed collected and handled by the Centre originates from natural forests. In 1988 over 14,000 seedlots from 600 species were dispatched, with more than 60% being sent overseas (Fig. 1).

All incoming seedlots are routinely fumigated for 2 weeks with carbon dioxide to restrict insect damage. Overseas dispatches are treated to meet the phytosanitary requirements of the recipient country.

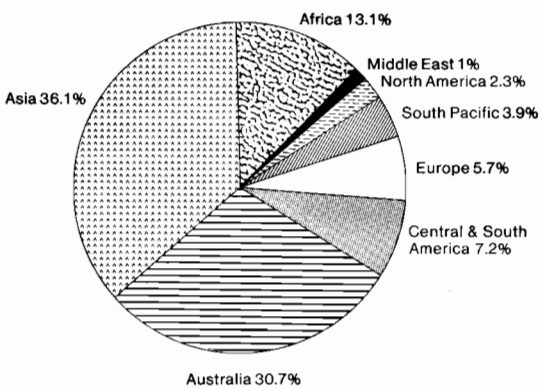

Fig. 1. Seed orders processed 1988; by geographic region.

There is, however, no seed pathology carried out at the centre's laboratory other than to note the growth of moulds on germination tests. Given the international role of the ATSC it was considered prudent to investigate possible fungal contamination.

Most seeds carry spores of various fungi either on the surface or within the tissues, and counts as

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high as 150,000 spores per tree seed have been reported (Anderson 1986). Some seed-borne fungi can cause the death of seeds (Gibson 1957). Other species generally viewed as being harmless can also cause serious losses. *Trichoderma* spp., for example which are normally considered to be saprophytic, can cause decay of seeds subjected to improper storage conditions (Anderson 1986).

Eucalypts grown as exotics are affected by many fungal pathogens. These are often indigenous organisms of wide host range that have become established on the introduced trees e.g. *Botrytis cinerea* Pers. ex Nocca & Balb. (Mittal et al. 1987), *Botryosphaeria ribis* Grossenb. & Dugg. (Barnard et al. 1987) and *Cylindrocladium* spp. (Anon. 1984a, b). There are however examples of leaf pathogens highly specialised on eucalypts, such as *Phaeoseptoria eucalypti* Hansford, *Aulographina eucalypticola* (Cooke and Massee) v. Arx and Muller, *Mycosphaerella nubilosa* (Cooke) Hansford and *Cercospora* spp., which have been recorded in several parts of the world on plantation eucalypts (Dick 1982; Anon. 1984a).

The most likely explanation for their presence is that they have been accidentally introduced on seed or other plant material. *Eucalyptus, Acacia* and *Casuarina*, three of the main Australian native tree genera, were selected for a systematic investigation of their seed mycoflora.

A review of available literature has shown that few mycological studies of seeds of these genera have been done either in Australia or overseas. Examples of such work are listed below:

Seven fungal species were isolated from seeds of *Acacia confusa* Merr. in the Philippines (Agmata 1979) and one species from seeds of *A. koa, A. koaia* and *A. confusa* in Hawaii (Richardson 1983).

Richardson (1979) listed *Pestalotiopsis* sp. in India and Mauritius, and *Phomopsis casuariniae* (Tassi.) Died. in India and Australia as seed-borne fungi of *C. equisetifolia* Forst. & Forst. Sahai and Mehrotra (1982) reported that 19 fungal species belonging to 11 genera were recovered from seeds of *Casuarina* in Nainital, India. Seed-borne fungi, especially *Penicillium* spp., associated with seeds of *Eucalyptus* spp., have been studied in the Philippines by Agmata (1979). Saxena (1985) detected 30 fungal species on seeds of *E. grandis* W. Hill ex Maiden and *E. tereticornis* Smith in Agra, India. Also in India, Mittal (1986) isolated 14 species of fungi from seeds of *E. tereticornis* and Tiwari and Sharma (1981) isolated a similar number of fungi from *Eucalyptus* sp.

Four potentially pathogenic species. (*Aspergillus niger, Fusarium* sp., *Penicillium canadense* and *Rhizopus oryzae*) were found on seeds of *E. citriodora* Hook. (Mittal and Sharma 1982).

The only previous study of mycoflora associated with stored seed samples of *Eucalyptus* spp. in Australia appears to be that of Mwanza and Kellas (1987). In that work, nine seed-borne fungi were isolated from *E. obliqua* L'Her. and *E. radiata* Sieber ex DC.

The aims of this study were to record fungi associated with seeds of *Acacia* spp., *Casuarina* spp. and *Eucalyptus* spp., and to determine the levels of infestation; to investigate relationships between the level of infestation and seed viability and to evaluate the pathogenicity of some commonly occurring fungal species. Other objectives were to detect possible influences of duration of storage on the level of infestation of seed, and to determine whether the standard fumigation treatment employed by ATSC had any influence on fungal contamination.

A total of 18 seed lots, (Table 1) were tested. With the exception of a single collection from Papua New Guinea (PNG), the seed was all collected in Australia. The seed was stored at the ATSC in Canberra.

Materials and Methods

Sampling

Working samples were prepared from submitted samples using the 'Mechanical Divider Method' and the 'Spoon' method as recommended by the International Seed Testing Association (ISTA 1976).

Superficial Contamination of Seed

Seeds were examined directly with a stereoscopic microscope at up to 60× magnification. Other working samples of 50 seeds of *Acacia* spp., 0.2 g of *Casuarina* spp., or 0.1 g of *Eucalyptus* spp. were shaken mechanically for 10 min with 10 ml water in a 'wrist action' shaker (Griffin Flask Shaker). Suspensions of particulate material were decanted from above the seeds and centrifuged for 5 min at 5000 rpm. Sediment in the tubes was examined for the presence of spores by means of a compound microscope and haemocytometer.

Mycoflora of Seed after Incubation

The presence of fungi and symptoms of disease on seeds or seedlings was determined by the 'Blotter Method' and by plating on potato dextrose agar (PDA) according to standard procedures of ISTA (ISTA 1976; Neergaard 1979; Sheppard 1979; Tempe and Binnerts 1979). Seed samples (25 seeds per dish for *Acacia*, 0.2 g seed for *Casuarina*, 0.1 g for *E. camaldulensis* and *E. grandis*, 0.2 g for *E. nitens* and 0.7 g for *E. globulus* ssp. *globulus*) were either surface sterilised with 1%

Table 1. Seed source of *Acacia* spp., *Casuarina* spp. and *Eucalyptus* spp. used in the study.

Sample number	Seedlot number	Species	Collection date	Origin: Location	Lat °S Deg	Lat °S Min	Long °E Deg	Long °E Min	Altitude (m)	Viable seeds/ 10g
1	15697	*A. auriculiformis*	1986	S of Coen, Cape York, Qld	14	7	143	16	160	540
2	16106	"	1987	3km N Mibini, PNG	8	49	143	38	40	268
3	16153	"	1987	Coopers Creek, NT	12	6	133	11	40	490
4	14637	*A. holosericea*	1984	E of Hooker Creek, NT	18	20	130	41	310	665
5	14927	*A. mearnsii*	1985	S Gippsland, Vic	38	0	147	0	100	686
6	13515	*C. cunninghamiana*	1982	9km SE of Mareeba, Qld	17	4	145	28	400	3600
7	14919	"	1985	Uriarra Crossing, ACT	35	14	148	57	420	12700
8	15958	*C. equisetifolia* ssp. *equisetifolia*	1987	Wangetti Beach, Cairns, Qld	16	41	145	34	0	1753
9	12187	*E. camaldulensis*	1977	8km W Irvinebark, Qld	17	24	145	9	680	6350
10	12964	"	1980	Emu Creek, Qld	17	20	144	58	460	9910
11	14338	"	1984	Region E of Petford, Qld	17	17	145	3	500	9800
12	15092	"	1985	Lake Albacutya, Vic	35	42	141	57	70	7410
13	15050	"	1985	Gibb River, WA	16	30	126	10	400	9800
14	16230	"	1987	Emu Creek, Qld	17	22	144	56	500	12075
15	13467	*E. globulus* ssp. *globulus*	1981	Police Point, Tas	43	15	147	5	250	540
16	13019	*E. grandis*	1980	NW of Coffs Harbour, NSW	30	13	153	2	135	8000
17	13289	"	1980	Mount Lewis, T. Res 66, Qld	16	36	145	16	1000	5370
18	16341*	*E. nitens*	1988	Errinundra Plat. Vic	37	16	148	51	1080	2710
19	16341	"	1988	Errinundra Plat. Vic	37	16	148	51	1080	2710

All seedlots listed here were fumigated with carbon disulphide and/or carbon dioxide before storage except for seedlot 16341*.
ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; Qld: Queensland; PNG: Papua New Guinea; Tas: Tasmania; Vic: Victoria; WA: Western Australia.

NaOCl aqueous solution for 4 min or rinsed in sterile distilled water (SDW), before being spread on blotters or plated on PDA, four replicates per seed sample.

Plates and blotters were incubated at 20°C for 8–12 days in alternating, 12 hours cycles of near ultra violet light and darkness. Fungal growth was recorded by microscopical examination, and the percentage frequency of seeds bearing each fungal species was recorded for each dish.

Seed Viability Test

Two methods were used to determine germination:

(1) Sand method Ten seed lots — 3 of *Acacia*, 2 of *Casuarina* and 5 of *Eucalyptus* — were selected and the same quantities of working sample as used in the seed mycoflora survey were tested in sterile, fine-grained sand. After being autoclaved at 120°C for 20 minutes, sand was placed into small-compartmented plastic punnets (10.5 cm^2). The sand was thoroughly pressed down and the surface was made even to a level 2 cm lower than the rim of the pots. Seeds of *Acacia* spp. were immersed in boiling water for one minute before being sown. All seeds were surface-sterilised with 1% NaOCl for four minutes and sown at 1-3 mm depth (depending on tree species), and a layer of coarse-grained sand (about 1 mm diam.) was sprinkled over them (10 mm deep for *Acacia* spp. and 5 mm for *Casuarina* spp. and *Eucalyptus* spp.). The pots were incubated in a glasshouse and watered automatically using a wet-leaf misting system. The emergence of seedlings was recorded after 40 days.

(2) Plating on PDA All 18 seed lots were plated onto PDA and incubated at 30°C, the optimum germination temperature. Germination was estimated daily from incubation day 3 to day 14.

Fumigation of Seed

A comparison was made of the mycoflora of seed fumigated with CO_2 before storage and that of nonfumigated seed. Seed of *E. nitens* collected in 1988 was divided into two lots and one was fumigated with CO_2 for two weeks before storage at 20°C. The other lot was not fumigated. Both samples were examined for the level of fungal contamination using the blotter and PDA plating methods.

Table 2. List of fungal species found in the seeds of *Acacia* spp., *Casuarina* spp. and *Eucalyptus* spp.

	Acacia spp.			*Casuarina* spp.		*Eucalyptus* spp.			
Fungal species	*A. auriculiformis*	*A. holosericea*	*A. mearnsii*	*C. cunninghamiana*	*C. equisetifolia* ssp. *equisetifolia*	*E. camaldulensis*	*E. globulus* ssp. *globulus*	*E. grandis*	*E. nitens*
Alternaria alternata				+		+		+	+
Aposphaeria sp.				+					
Aspergillus niger	+	+		+	+	+	+	+	+
Aspergillus sp.	+		+	+				+	
Botrytis cinerea					+				
Cephalosporium sp.				+		+	+		+
Chaetomium cochlioides	+					+	+	+	
Chaetomium sp.				+					
Cladosporium cladosporoides				+					
Cladosporium sp. (type 1)						+			
Cladosporium sp. (type 2)			+						
Cunninghamella sp.					+				
Curvularia lunata	+					+		+	
Curvularia senegalensis	+				+	+			+
Cytospora sp.						+		+	+
Drechslera spicifera						+			
Epicoccum purpurascens	+					+		+	+
Fusarium solani		+							
Fusarium sp.	+					+			
Gliocladium roseum						+			
Helminthosporium sp.			+						
Memnoniella echinata						+			
Penicillium frequentans						+		+	
Penicillium sp. (type 1)				+	+	+		+	+
Penicillium sp. (type 2)							+		
Penicillium sp. (type 3)							+		
Penicillium sp. (type 4)	+		+	+		+			+
Pestalotiopsis sp.	+			+	+				
Phoma sp.				+		+	+	+	+
Rhizopus stolonifer				+		+	+	+	
Sporormiella australis		+	+						
Stachybotrys chatarum									+
Trichoderma sp.				+		+	+		+
Trichothecium roseum				+	+				
Ulocladium sp. (type 1)						+			
Ulocladium sp. (type 2)									+
Ulocladium sp. (type 3)	+	+	+	+	+				
Unknown isolate	+				+				

Pathogenicity Tests

The pathogenicity of 14 of the most commonly isolated fungi, excluding certain moulds such as *Penicillium, Aspergillus* and *Rhizopus* spp. that were not putative pathogens, was tested. Seedlots of *A. auriculiformis* Cunn. ex Benth, *C. cunninghamiana* Miq., and *E. camaldulensis* were used.

Seeds were sown into pots containing autoclaved sand and inoculum prepared by suspending in SDW spores from 1-month-old cultures of fungi grown on PDA. Suspensions were strained through double layers of sterile cotton gauze and their concentrations standardised to approximately 10×10^5–10×10^6 spores per ml. With the exception of *Cladosporium cladosporoides* (13%), *Chaetomium cochloides* (18%), *Curvularia lunata* (41%) and *Trichothecium roseum* (48%), all fungi recorded spore viabilities of more than 50%.

Seeds of the three tree species, spread on the sand, were sprayed with the inocula using a hand atomiser (Camag Unispray, Mutten Switzerland). Seeds were covered with coarse-grained sand and the pots were incubated in a growth cabinet at 25°C for 12 h light, and 18°C for 12 h dark. They were watered once a day with demineralised water. Observations were made at intervals up to day 60 of incubation and seedlings categorised as follows: normal; wilted or damped-off; blighted (germinated but no emergence); non-germinated. Seedling height and root length were measured after harvest to determine whether any of the fungi influenced post-emergence growth. Controls were uninoculated seeds in autoclaved sand.

Results

Examination of Ungerminated Seed

The following fungi were found growing on seeds or associated plant debris by direct microscopical examination.

Approximately 1.6% of a sample of *A. auriculiformis* collected in PNG bore mycelium of a *Fusarium* sp. Seeds of *C. equisetifolia* ssp. *equisetifolia* from Cairns, Queensland and leaf fragments of *C. cunninghamiana* from the Australian Capital Territory bore pycnidia of *Pestalotiopsis* sp. with an infection frequency of 8.1 and 11.8% respectively. Pycnidia of *Phoma* sp. were found on chaff with seeds of Queensland samples of *E. grandis* (0.08%) and *E. camaldulensis* (0.03%).

Pestalotiopsis sp. and *Ulocladium* sp. were found in seed washings of all three tree genera, and *Phoma* sp. was observed from *C. cunninghamiana* and several eucalypt species. Other fungi detected in seed washings were *Curvularia lunata, Drechslera spicifera* and *Penicillium* sp.

Fungi on Seed Incubated on Blotters and Plates

A total of 250 fungal isolations were made, 57 from *Acacia* spp., 44 from *Casuarina* spp. and 149 from *Eucalyptus* spp. Twenty five fungal genera including two Zygomycotina, two Ascomycotina and 21 Deuteromycotina, representing at least 38 species were recovered from the 18 seed lots. Table 2 lists the total number of species of fungi isolated in these studies.

Fungi of 12 genera were recovered from more than 10% of the seed of one or more seedlots. These genera and the maximum frequencies of their recovery from the 9 native tree species are shown in Fig. 2. The fungi most commonly observed were saprophytic 'storage fungi' (Neergaard 1979) including *Aspergillus, Penicilium, Alternaria*, and *Rhizopus* spp., and some seed samples were very heavily contaminated with these genera.

A comparison between the level of fungal contamination and time in storage was made for six

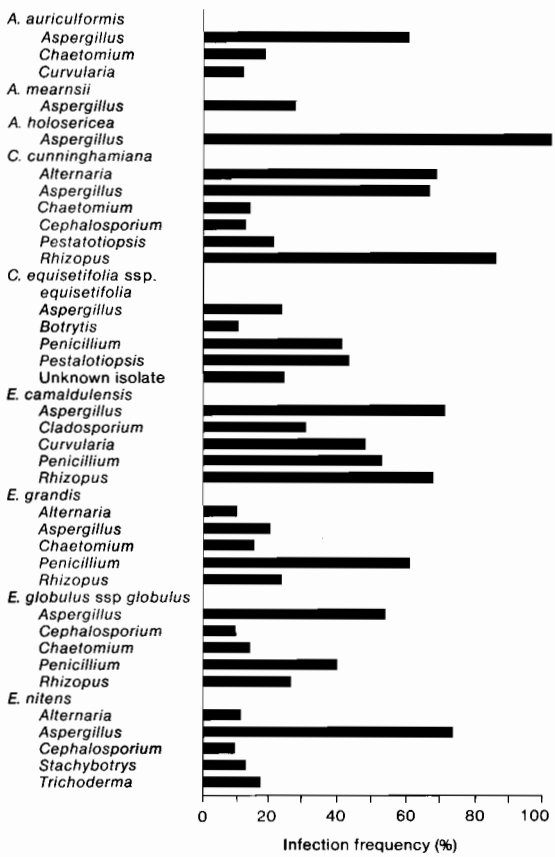

Fig. 2. Common fungi (with the maximum infection frequency of more than 10%) associated with seeds of *Acacia* spp., *Casuarina* spp. and *Eucalyptus* spp. on blotters and PDA.

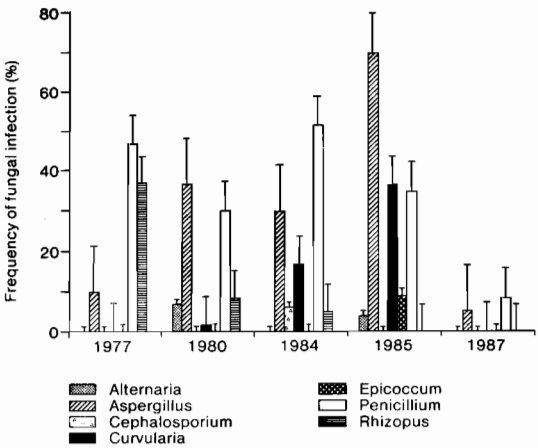

Fig. 3. Infection frequency of common seed-borne fungi (with the maximum infection frequency of more than 5%) associated with seedlots of *E. camaldulensis* collected at the different year and different parts of Australia on blotters.

seed lots of *E. camaldulensis*. This was not a true reflection of storage time only, as the seed was collected at different locations and at different locations over a period of 10 years.

There was no evident effect of storage period, either on the overall level of contamination or on the presence of 7 of the most commonly isolated fungal genera (Fig. 3).

Similarly, fumigation of a seedlot of *E. nitens* had no effect on the recovery of fungi on blotters and PDA, but the treatment reduced the spectrum of isolated species by approximately one half compared to nontreated seeds.

Fungal Contamination and Seed Viability

Presence of fungi on germinating seedlings was recorded for species of *Eucalyptus, Acacia* and *Casuarina*, with and without surface sterilisation. Viability was tested both on sand and on agar. Germination was increased by surface sterilisation using both tests (Table 3), however even with the agar germination test there was only a weak negative correlation between the frequency of seed germination and the level of fungal infection. Contamination above a level of 60% did not result in further depression in germination frequency.

Pathogenicity of Fungi Isolated from Seed

Fourteen fungi isolated from seed were selected as putative pathogens and were tested for their

Table 3. Percentage seed germination and the total infection frequency by fungi in *Acacia* spp., *Casuarina* spp. and *Eucalyptus* spp. on PDA and sterile sand.

		On PDA				On sterile sand			
		N.S.S.		S.S.S.		N.S.S.		S.S.S.	
Seedlot No.	Tree species	germination	frequency	germination	frequency	germination	frequency	germination	frequency
15697	*A. auriculiformis*	51.0	9.0	79.0	0.0	67.0	30.0	70.0	20.1
16106	"	22.0	32.0	41.0	0.0				
16153	"	22.0	64.0	57.8	13.2				
14637	*A. holosericea*	77.0	13.0	58.0	0.0	80.0	0.7	81.3	0.0
14927	*A. mearnsii*	89.0	14.0	97.5	0.0	96.0	6.0	98.0	0.3
13515	*C. cunninghamiana*	14.4	99.0	48.5	12.1				
14919	"	14.8	99.6	36.2	0.0	44.0	28.8	65.0	0.0
15958	*C. equisetifolia* ssp. *equisetifolia*	7.8	84.5	21.7	18.6	69.0	2.1	86.0	4.5
12187	*E. camaldulensis*	41.3	48.7	98.1	14.6				
12967	"	31.7	80.0	59.1	0.0				
14338	"	39.3	99.4	80.5	10.2	60.0	1.6	88.0	0.0
15029	"	18.5	53.3	95.0	2.1				
15050	"	41.3	73.4	58.7	40.0	79.0	0.0	89.0	0.0
16230	"	18.6	75.3	76.6	0.0				
13467	*E. globulus* ssp. *globulus*	32.1	98.2	98.5	15.0	85.0	36.4	89.0	44.0
13019	*E. grandis*	37.5	91.6	84.3	15.2				
13289	"	51.2	20.4	98.4	0.0	73.0	0.0	85.0	0.0
16341*	*E. nitens*	52.3	83.5	81.2	0.0				
16341	"	45.2	93.5	79.5	2.0	97.0	0.4	99.0	2.1
Mean		37.2	64.7	71.0	7.5	75.0	10.6	85.0	7.1

The values are the total infection frequency by all fungi appearing in the PDA test and in the sterile sand test. Counted after 14 days incubation in PDA test and 40 days in sterile sand test.
S.S.S.: Surface-sterilised seeds; N.S.S.: Non-surface-sterilised seeds.
*The seedlot was not fumigated before storage.

Table 4. Percentage emergence of seedlings of *Acacia auriculiformis*, *Casuarina cunninghamiana* and *Eucalyptus camaldulensis* after inoculation with 14 species of fungi.

Fungus inoculated	*A. auriculiformis*	*C. cunninghamiana*	*E. camaldulensis*
Alternaria alternata	71.1 cde	64.6 de	39.6 cde
Aspergillus niger	72.9 cd	54.2 f	40.0 cde
Botrytis cinerea	69.7 de	59.9 ef	53.6 ab
Chaetomium cochlioides	78.4 bcd	68.8 bcd	48.3 bc
Cladosporium cladosporoides	78.2 bcd	64.6 de	46.3 bcd
Curvularia lunata	69.7 de	55.7 c	42.0 cde
Curvularia senegalensis	62.5 ef	71.1 abcd	29.3 f
Cytospora sp.	79.9 bc	65.3 cde	52.4 ab
Drechslera spicifera	53.2 g	40.1 g	35.8 ef
Fusarium solani	24.8 h	28.2 h	35.3 ef
Fusarium sp.	55.5 fg	73.7 ab	29.1 f
Pestalotiopsis sp.	56.7 fg	63.2 bcd	37.5 def
Phoma sp.	90.2 a	70.3 abcd	41.0 cde
Trichothecium roseum	84.9 ab	73.5 abc	42.8 cde
Control	86.7 ab	78.0 a	57.1 a

Counted after 60 days incubation.
Values sharing a same superscript in each vertical column are not significantly different ($p < 0.05$), according to Duncan's multiple range test.

capacity to cause disease in germinating seed and seedlings, using a standard method. Table 4 shows the seedling emergence for *A. auriculiformis, C. cunninghamiana* and *E. camaldulensis* compared to control, non-inoculated seeds.

For *A. auriculiformis, F. solani* caused the maximum reduction in germination of seed, followed by *D. spicifera, Fusarium* sp., *B. cinerea, C. senegalensis, Pestalotiopsis* sp. *C. lunata, A. alternata, A. niger* and *C. cochlioides*. Inoculated fungi were readily isolated from rotted seed. *F. solani, Fusarium* sp. and *Pestalotiopsis* sp. also caused between 3 and 6% post emergence damping-off and wilting of seedlings. For *C. cunninghamiana, F. solani, D. spicifera, A. niger, C. lunata, B. cinerea, A. alternata, C. cladosporoides, Cytospora* sp., *C. cochlioides* and *Pestalotiopsis* sp. caused significant pre-emergence seedling blight. Only *Pestalotiopsis* sp. was associated with significant post-emergence seedling death.

Of the 14 fungi inoculated on to *E. camaldulensis* seed, all but *B. cinerea* and *Cytospora* sp. reduced seedling emergence. Again the *Fusarium* spp. were the most pathogenic and *F. solani* (6%) together with *C. lunata* (2.3%) also caused damping-off of emerged seedlings. Measurements of seedling growth after emergence showed that the height and root length of surviving seedlings were not significantly affected by any of the inoculated fungi.

Discussion

This work has shown that the seedlots selected from the ATSC were contaminated with a wide range of fungal species and that the standard fumigation treatment carried out prior to dispatch has little effect on the level of contamination.

The most commonly found fungal species were storage moulds sensu Neergaard (1979), ubiquitous on stored seed of most crop plants. These reduced the viability of seed after sowing, as shown by the seed viability tests coupled with surface sterilisation treatments. However, their effect was most marked when seeds were germinated on agar. Tests on sterilised sand showed only a small effect on viability even with seedlots shown to be very heavily contaminated with fungi (Table 3). It seems likely, therefore, that contamination with storage moulds would have a small effect on seed sown in sterilised soil mixes under commercial conditions. According to the available literature, 11 of these fungal species appear to be new records for seeds of the genus *Acacia*, 6 for *Casuarina* and 10 for *Eucalyptus*. Several of the fungi recovered from seedlots belong to genera that include pathogens, or are known to be pathogenic in their own right, e.g., *F. solani, D. spicifera, Curvularia lunata* and *Phoma* sp.

These fungi, together with other species not likely to be significant pathogens (*Aspergillus niger, Cladosporium cladosporioides*), caused seedling blight and reduced emergence when they were artificially inoculated at high dose rates onto seed placed to germinate on sand. The test gave an acceptable indication of pathogenicity, as recognised seedling blight pathogens such as *Fusarium* spp., *Curvularia* spp., *Drechslera spicifera*, and *Pestalotiopsis* sp. consistently caused most disease on all three tree genera.

The fungal pathogens recovered from seed or seedlings in this study are already widely distrib-

uted geographically and do not pose a quarantine threat to countries receiving shipments of seed from ATSC. The fact, however, that so many fungal species, including several pathogens were readily observed on seed that had been collected and stored under the strict conditions imposed by ATSC implies a potential for more serious pathogens to be disseminated in this way. Of particular note were direct observations of fructifications of pathogenic fungi on the seed coats of *A. auriculiformis* from PNG and leaves or chaff accompanying seed of *Casuarina* and *Eucalyptus* from Queensland.

Certain measures can be adopted to minimise the risk of spread of disease in seedlots, especially small amounts dispatched overseas to form the basis of planting initiatives. Chaff and leaf fragments appear to be particularly hazardous and every effort should be made to sieve these from samples. Surface sterilisation prior to sowing not only may increase germination by reducing the load of storage fungi on the seed but may also remove pathogens. During these studies there were some instances where recovery of certain pathogenic fungi was enhanced by surface sterilisation, presumably as a result of removal of antagonistic fungi. Among suggested surface treatments (Donald and Lundquist 1984) are hot water (50°C), 10% sodium hypochlorite, and fungicides. *Acacia* seed is routinely treated with boiling water before sowing to promote germination (Doran and Gunn 1987). This would also remove some fungal contaminants.

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Country Reports

An Overview of the National Tree Seed Centre, Papua New Guinea

N.H.S. Howcroft*

Abstract

This paper contains a brief history of the establishment of the National Tree Seed Centre at Bulolo, Papua New Guinea, which was built with funds from the New Zealand Overseas Development Agency and opened in November 1987. It also describes the Centre's main functions in seed collection and processing, from the viewpoints of research and development, education and training, and industrial and commercial enterprises.

THE National Tree Seed Centre (NTSC) is a recently established unit managed by a field staff comprising one senior professional officer and three technical officers of the new Papua New Guinea Forestry Research Institute, Lae. All the staff have had previous experience with tree improvement, seed procurement and production. The Centre is located at Bulolo where it is supported by the National Seed Production Area (NSPA) and year-round seed collection activities. (Fig. 1).

The Seed Centre, and its equipment, was donated by the Government and people of New Zealand. The project was funded through the New Zealand Overseas Development Agency; construction started in 1986 and the Centre was officially opened in November 1987.

In this paper a brief history of the establishment of the Centre is provided and the role and the scope of the Centre are outlined.

History

In the past, seed procurement and storage in Papua New Guinea — for reforestation, afforestation, extension and research projects — were supported by two seed stores. These were maintained by the National Forestry Department at Port Moresby and Bulolo. Whilst the Port Moresby seed storage unit mainly handled seed in transit, the Bulolo unit was the more important, as it was the major distributor of seeds to most of Papua New Guinea. More importantly, it was located where seed procurement operations were the most active, and from where provenance research projects, tree improvement projects and seed orchard development projects were being conducted by the Bulolo Research Field Station.

In the latter part of the 1970s, the Bulolo reforestation project and the Bulolo seed store became the responsibility of the Morobe Provincial Government. The seed store went through a bad period and a lack of maintenance resulted in a number of cool room failures and a serious loss of seed, including valuable research seed. There was no indication that this problem would be solved and, as a consequence, aid was sought and received to establish an independent unit for the storage of seed for industry and research.

The old Bulolo seed store is now used as a backup unit. Its seed extraction area, designed to dry *Araucaria* cones and extract seed, is still used for this purpose. However, this seed is now stored in the NTSC and only a few seed samples of some species are stored in the one remaining room still functioning in the old unit.

* National Forest Research Field Station, Bulolo, Papua New Guinea.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Acacia angustissima	●							●	●	●	●	●
Acacia aulacocarpa												
Acacia auriculiformis								●	●	●		
Acacia mangium					●				●		●	
Agathis alba	●	●								●	●	●
Albizia falcataria ssp. *falcataria*	●	●				●						●
Albizia falcataria ssp. *fulva*	●	●										
Anthocephalus			●	●								
Araucaria cunninghamii									●	●	●	
Araucaria hunsteinii								●	●	●	●	
Calliandra calyothrysus	●	●	●					●	●	●		
Calliandra houstoniana	●	●	●					●	●			
Coffee		●	●	●	●	●						
Eucalyptus deglupta	●	●	●	●	●	●	●	●	●	●	●	●
Eucalyptus tereticornis					●	●						
Eucalyptus torelliana					●	●	●	●		●		●
Eucalyptus urophylla				●			●	●	●	●	●	
Gmelina arborea	●						●	●	●	●	●	●
Leucaena (all species)	●	●	●	●	●	●	●	●	●		●	
Octomeles		●										
Pinus caribaea var. *caribaea*	●	●	●									
Pinus caribaea var. *hondurensis*	●	●	●	●	●							
Pinus merkusii		●	●	●	●	●	●				●	
Pinus oocarpa			●			●			●	●	●	
Pinus strobus var. *chiapensis*				●	●							

Fig. 1. Guide chart to seed collecting (month of collections are indicated by dots)

Facilities

The Seed Centre is a single cement brick building with an office-reception room, an airconditioned laboratory with bathroom and toilet, an airconditioned seed store room with two coolroom units, a seed processing room and an emergency standby generator room with a generator to cope with power failures which are a common occurrence. The NTSC is equipped with microscope, balances, moisture meter, containers, seed extraction and cleaning equipment. Some items of seed processing and testing equipment have yet to be received.

Role and Scope

The NTSC has been in operation since its official opening in 1987 and activities, such as seed collecting and processing operations, storage and dispatch, have been increasing. Staff will require further training in various aspects of seed technology, research, management and administration, with the aim of continuously upgrading standards. When fully functional the NTSC's primary objective will be to meet national and international requirements for a reliable supply of certified seed of both indigenous and exotic species. The program is being expanded to include most genera of potential multipurpose tree species.

The Centre will be responsible for four main areas of activity — research and development, education, industry and commerce. Within the framework of these will be greater emphasis on the conservation, development and utilisation of indigenous genetic resources.

Research and Development

The unit has the responsibility of exploring native forests and collecting seed from indigenous genetic resources, also maintaining, evaluating, documenting and distributing genetic material from Papua New Guinea. Exploration of natural stands of potentially useful tree species in the country, and seed collection from these, is sup-

ported by FAO funding; this work goes back to the latter part of the 1970s (Boland et al. 1977; Howcroft, 1977, 1978, 1980, 1988; Skelton, 1987; Skelton and Howcroft 1987). The unit is also responsible for the establishment and evaluation of provenance seed production areas, some of which are classified as ex situ conservation stands as the original sources have been seriously depleted by logging and agricultural development. Papua New Guinea's flora is rich and diverse in many unevaluated species and, in the future, seed collecting will also include many other indigeneous species that may prove to be economically and socially important as multipurpose species.

Provenance and tree improvement research will be supported by the NSTC. This work is ongoing with *Araucaria, Eucalyptus* and *Pinus*, but it is being expanded to include *Acacia* and others such as *Leucaena.*

Seed technology research is also a responsibility of the Centre and will be required for many of the indigeneous species, as little is known about the processing and storage requirements of many of these species. One of the initial aims of seed technology research will be to obtain maximum longevity and germination capacity in storage and during transit. Such work will support lowland rainforest research and management where seed will be required for enrichment or for gene pool conservation. The current level of activities in this work is low at present, but it will be stepped up in the next few years as staff gain training and experience in seed technology. The Forestry Research Institute will provide additional support for this work.

Education and Training

The research station's facilities and the staff's expertise have been used in the past by public schools, the Bulolo Forestry College and the University Department of Forestry, Lae, for the education and training of their students. The NTSC will be used similarly in the future. The Centre is accessible by road from Lae in a few hours and by air from other areas.

Industry

As mentioned earlier, the main objective of the NTSC is to satisfy the local and international seed requirements through the supply of certified and well documented seed of quality. As the quality and the kind of species produced is dictated to the seed producer by the industry, the Seed Centre aims to meet these demands and remain flexible in terms of species availability, both in the exotic and indigenous lines.

Most seeds for plantation establishment, required by all the major government and private sector plantation projects in Papua New Guinea, are supplied from the NTSC's clonal and seedling seed orchards or seed production areas and plantation seed trees. The only exceptions to this are those provincial forestry departments in the highlands who draw their supplies of *Eucalyptus* and *Pinus patula* seed from their plantations and from the *Pinus patula* seedling seed orchard, which was established by the research section for this purpose.

Commerce

The high cost of collecting seed from natural stands, and the cost of local seed production, processing and storage, dictate that the National Tree Seed Centre must be commercially oriented in much of its activities. This does not include seed for research, and seed exchange will continue.

Seed procurement and seed production activities in Papua New Guinea are taking into account the international market's need for seed of Papua New Guinea species. At present it has been possible to make available only limited amounts, but the growing demand for certain species has prompted the Seed Centre to expand this area of seed production at Bulolo and elsewhere.

Commercial seed distributed by the Centre will be priced according to the cost of collection, processing and storage. Professional seed collectors will appreciate that species collected from natural stands can be expensive. This is certainly the case in Papua New Guinea, due to the high cost of travel and difficulties such as the acquisition of local transport, labour, lack of data on the phenology of the species, poor crops and the cost of extracting and bringing the seed back.

The National Seed Production Area

The need for permanent seed sources of exotic and indigenous tree species in Papua New Guinea was recognised in the 1950s. Since then programs have been initiated to establish and develop seed sources of important plantation species. The establishment and continuing development of a national seed production area at Bulolo is one of these programs.

This area is located 0.5 km north of the National Tree Seed Centre, latitude 7° 11′30″S, longitude 146°39′E, at an altitude of 700–750m. The mean annual rainfall is around 1600 mm per annum. The months of low rainfall are between May and September (MacAlpine et al. 1975.). The climate and site is suitable for many lowland species and in some instances, such as with *Eucalyptus deglupta*, flowering and fruiting may be more prolonged than on the coast.

Table 1. Species accessions established as seed sources.

Species	Provenances established	
	No. in NSPA	No. of ancillaries
Acacia auriculiformis	1	2
aulacocarpa	2	1
angustissima	2	—
cincinnata	1	—
crassicarpa	2	1
mangium	3	1
polystachya	—	1
Agathis alba	2	—
labillarderii	2	—
robusta	1	—
macrophylla	—	1
Albizia (Paraserianthes)		
falcataria	2	—
chinensis	1	—
Allocasuarina littoralis	1	—
Anthocephalus cadamba	2	—
Araucaria cunninghamii	1 clonal	3 clonal 5 PSA
hunsteinii	1 clonal	1 clonal 1 SSO
Calliandra calothrysus	1	1
houstoniana	1	1
Castanospermum australe	1	—
Casuarina grandis	1	—
Cordia alliodora	4	—
Eucalyptus alba	1	—
deglupta	2 clonal 2 SSO 2 PSA	1 PSA
pellita	2	—
raveretiana	1	1
tereticornis	1	2
torelliana	1	1
(torelliana × citriodora)	1	—
urophylla	1	2
Gmelina arborea	7	—
Gymnostoma papuana	1	—
Leucaena colinsii	1	—
diversifolia	2	—
aff. diversifolia	1	—
lanceolata	1	—
leucocephala	10	—
macrophylla	2	—
pallida	1	—
Pinus caribaea var *hondurensis*	1 clonal	1 clonal 2 SPA
merkusiana	6	2
merkusii	1 clonal 3	1 clonal —
oocarpa	14	8
tecunumannii	5	1 clonal 3
Terminalia brassii	4	—
complanata	1	—
impediens	1	—

Table 2. Acacia provenance seed production areas in Papua New Guinea, August 1989.

Location	Species	Provenance	Estab.	Refilled	Area (ha)
Bulolo	*A. auriculiformis*	Bula, W.P.	1984	—	1.05
		Iokwa, W.P.	1984	—	0.98
		Balamuk, W.P.	1984	—	1.96
	A. aulacocarpa	Oriomo, W.P.	1987	1988	0.75
		Iokwa, W.P.	1987	1988	1.81
		Keru, W.P.	1988	—	0.29
	A. crassicarpa	Wiroi-Wipim W.P.	1985	1989	2.78
		Wemenever W.P.	1985	—	2.28
		Mata, W.P.	1986	1988	1.25
		Oriomo, W.P.	1987	1988	1.50(?)
	A. mangium	Oriomo, W.P.	1985/89	1989	1.11
		Balamuk, W.P.	1986	—	0.64
Madang	*A. mangium*	Iokwa, W.P.	1984	—	4.00
		Balamuk, W.P.	1983	—	4.00
		Toko, W.P.	1984	—	4.00
		Oriomo, W.P.	1985	—	4.00

Note: All provenance seed production areas are to be expanded as further collections are made for each provenance. W.P. = Western Province.

Some 66 species of trees and around 156 sources have been established in the NSPA at Bulolo — for trials, conservation, breeding and seed production. A short list of some of these species is provided in Table 1. Clonal orchards of *Araucaria cunninghamii, A. hunsteinii, Pinus caribaea* and *P. merkusii*, are located in the NSPA. Room has been left to expand these in the future. Because of the need to isolate provenance seed production areas for some species, such as for the *Acacia*, it has been necessary to establish some of these in other parts of the Bulolo forestry plantation.

Current Programs

The NTSC has a current program of exploration of forest genetic resources and seed collecting. This is considered an ongoing program and at present involves the collection of seed of local *Acacia* species and *E. deglupta* from different localities in Papua New Guinea (Skelton 1987; Skelton and Howcroft 1987). In connection with this, the NTSC is actively establishing provenance seed production areas of *Acacia aulacocarpa, A. auriculiformis, A. crassicarpa,* and *A. mangium.*

Expansion of these areas is to continue as new collections of each provenance come to hand. The program has allowed for future tree improvement work with the *Acacia* species and to date all families, with each species and provenance, can be identified in the field. A list of these is provided in Table 2.

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Seed Improvement of Multipurpose Tree Species in Nepal, with Special Reference to Forage Species

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Abstract

Seed improvement in Nepal is only just getting underway. The species selected for improvement are mainly pioneer and timber species and the need to choose a different set of selection criteria from those already developed for timber species has not arisen. However, there is a pressing need to apply improvement techniques to other species, most of which are multipurpose. Selection criteria for multipurpose tree species are only vaguely defined in the literature, and as far as can be ascertained, have yet to be applied in practice. Purely mechanical selection methods will be preferable until sufficient work has been carried out to justify other selection criteria, so that the widest genetic base can be retained, and loss of desirable characters avoided.

A BEGINNING has been made in Nepal in the process of selecting stands for seed production, and the second phase, that of demarcation and opening of the crowns to encourage seed production, is about to commence. The species selected for this treatment so far are all multipurpose to a degree, but their primary use is as pioneer species with later use for timber production. The question of which desirable characters to select is not difficult for timber plantations. Site preference and the established good timber characters are quite adequate as long as the secondary uses remain relatively unimportant.

However, where thinnings or the final crop have important secondary uses, the position becomes more complex. These pioneer species remain of primary importance because sites available for plantation establishment are generally degraded, and will not support growth of the more desirable multipurpose species which the rural population prefers. Secondary planting of these more desirable species is the major route to their establishment. Whereas the pioneer species are relatively few in number the number of multipurpose tree species is legion, each with its advocates either rural producers or professional foresters. The major utilisation classes for multipurpose tree species within Nepal are forage, fuelwood, timber and food, possibly in that order; many other uses such as honey production can also be cited. A first look at selection criteria for multipurpose tree species shows a very wide range of possible characters as well as their probable conflicting nature when selection for more than one purpose is in mind.

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Range of Utilisation

Where multipurpose tree species are required for their physical or physiological characteristics in addition to one major product, e.g. as shade trees, controlling erosion or as nitrogen-fixers, there is no great loss of production in the major role when characteristics for the secondary role are selected in an improvement program. Where, however, the secondary role involves a significant division of the available biomass or a loss of productive capacity, as with fodder species, the primary production in the major role is affected to an extent not easily predicted. Fodder, fuelwood and

resin collection, fruit and seed production, bark renewal etc. all present a drain on both the excess carbohydrate available and the mineral balance of the tree, but fodder collection, particularly when carried out early in the foliage cycle (Table 1), causes a reduction of the subsequent total growth potential, and removes mineral nutrients in quantities that the tree can only recover over a considerable period.

Table 1. Important early and late lopped fodder species in Nepal.

Stage of growth giving best quality fodder	
Young foliage — early lopped	Old foliage — late lopped
Castanopsis spp.	*Ficus auriculata*
Ficus auriculata	*F. nemoralis*
F. lacor	*F. semicordata*
Morus alba	*Litsea monopetala*
Schima wallichiana	

Conflicting Aspects of Production

The major factor within the tree controlling crop production rate is dry matter production and its distribution and utilisation. Dry matter production is dependent on leaf area and any reduction in this component will adversely affect the rate at which dry matter becomes available for conversion into the desired product. A major factor controlling distribution of the available photosynthate is flowering and fruiting, which preferentially diverts and utilises material that would otherwise go into wood, new leaf or resin. Although it is possible for a tree to provide more than one product, and perfectly normal for it to do so, the rate of production of the major product will normally be adversely affected by the production of the second. Successful improvement programs have relied on a reduction of secondary products in order to enhance production of the single target crop. Quantitative aspects like these are dependent on the strengths of the various 'sinks' to decide the balance of the eventual crop production.

For many purposes the concept of a multipurpose forest would be more easily pursued than that of the multipurpose tree even for quite small village plantations. Trees could then be selected for superior characters in one main product, with possibly a second of minor importance. This would be the most easily obtainable goal for the present in Nepal, otherwise the multiplicity of species and characters would make for very slow progress in seed improvement.

Fodder Production and Quality

Tree leaves are used for animal feed largely in the dry, winter season from October to May, when forage is in very short supply, i.e. the animals cannot feed themselves. A very wide range of species is utilised, and there are strong regional preferences, reflecting farmers' experience in maintaining animals in good condition. Two aspects that need to be considered are the prime time for collection in terms of feed value, and the best time for collection in terms of the ability of the tree to regenerate foliage or enter a normal seasonal rest period, and the subsequent effects on tree growth and production. Both fields appear to be wide open for productive research.

Improvement of Single Purpose Fodder Species

It has recently been proposed that the tree improvement program for Nepal should include fodder species either as single or multipurpose ones. A brief discussion of our present knowledge of the fodder trees of Nepal will help highlight the factors which must be considered before such a process can begin.

In the first place complexity of species numbers, ecological range and utilisation patterns makes selection of a reasonable number of species a difficult task. It is necessary to include in any program species that will grow and produce fodder on a very wide range of sites, stratified on the basis of precipitation, altitude and soil type. In addition it is becoming increasingly clear that there is often a wide range of fodder species within a very small area. Robinson and Thompson (1988) report on an area of three wards within one panchayat of a hill district where 70 species of trees and shrubs are currently utilised for fodder. Another study in this series, conducted on both sides of a hill with an altitudinal range of 1000 m, found that farmers, asked to name the five best fodder species, included 70 species between them. In order to reduce this large number of species to a few high quality ones on which improvement could begin, two approaches have been explored; firstly, evaluation based on farmer knowledge and secondly, formal experiments and scientific evaluation.

Evaluation Based on Farmer Knowledge

The reason why lists of preferred species are so long and variable is that each farmer uses only a small number of the total species available, and his knowledge is restricted to these few. In addition, preference rankings are influenced by attributes such as fodder quality, yield, and sea-

sonal availability, regrowth potential and number of possible harvests a year. These attributes will vary in their relative importance to a farmer depending on his own situation. A farmer with no shortage of fodder will rank a list mainly on the basis of quality, whereas one with a fodder shortage may put a higher value on yield or availability at a particular season.

During the course of these surveys an added problem has been one of precise definition of fodder trees by name because of varietal differences within species which have not yet received sufficient botanical examination or which have ecotypes which are not morphologically distinct but exhibit well marked differences in terms of fodder quality. Examples are *Artocarpus lakoocha, Bauhinia variegata* and four species of *Ficus,* one of which, *Ficus semicordata*, has three well marked varieties known as Khasro-, Rai-, and Lute-Khanyu. Not all farmers are aware of these variations in quality and may rate a species much lower than it otherwise deserves on the basis of its best forms.

The production of valuable materials other than fodder may also influence a farmer's ranking, as well as other less obvious factors such as social and religious connections.

Nevertheless, although there are a number of problems with using farmer's 'knowledge' in selecting species on which more research should be concentrated, if surveys are carefully conducted by people who have experience with the way in which farmers evaluate trees, valuable information can be provided.

Formal Experiments and Scientific Evaluation

It is obvious from the previous section that the complexity of growth periodicity, yield and quality would demand very careful field and laboratory examination for a decision on selection criteria for fodder species to be properly arrived at. In addition, the assessment of yield in fodder trees is highly complicated as the discussion in Robinson and Thompson's (1988) paper brings out. What is even more difficult to resolve is the assessment of fodder quality, particularly on the basis of chemical analyses. Composition varies with season, location within the tree canopy and fodder components. Even assuming that a proper sample of leaf and edible stem can be obtained from which to analyse the digestible constituents, the chemical methods available do not give meaningful results in terms of true feed value to animals (Robinson 1986, Robinson and Thompson 1988). This is because of the range of secondary compounds such as phenolics and alkaloids in tree leaves, which either inhibit digestion or which are toxic to some degree. A further complication is that different types of animal are able to digest tree fodder with differing efficiency.

Conclusions

The production of improved seed in forest trees is achieved through a number of steps which follow in an orderly series. Firstly, opening of the crowns in selected stands will normally improve the quantitative aspects of seed production. Secondly, selection of desired characters and the elimination of undesirable or unwanted characters in seed production stands leads to improvement in seed quality. For timber production the selection criteria are well tried and proven, but for other end-uses this is not the case.

For fodder species several problems have to be solved before selection criteria can be tested. These problems include taxonomic determination, reduction of species multiplicity and evaluation of fodder quality. Once these problems are solved a start can be made on the determination of appropriate selection criteria for fodder, and these will necessitate a consideration of silvicultural, physiological, biochemical and animal husbandry factors. Species harvested for fodder early in the leaf production cycle are unlikely to have the dry matter production capacity or mineral nutrient status to function effectively as multipurpose tree species.

Until this process has been completed, thinning of seed production stands should be purely mechanical with no element of human selection beyond the elimination of obviously unsuccessful individuals. This should apply to all end-uses other than timber production. Those engaged in seed improvement work with multipurpose tree species could thereby begin the process, improve the quantitative aspect of seed supply, satisfy the demand for progress and at the same time avoid qualitative pitfalls leading to seed deterioration rather than seed improvement.

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Germplasm Exchange of Multipurpose Trees: an Indian Perspective

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Abstract

Exchange of plant genetic resources has greatly benefited agriculture and forestry programs all over the world. In this process, we have also learnt to be rigorous about quarantine. It is essential for every country to have an efficient quarantine system so as to safeguard against entry of new pests and pathogens during exchange of germplasm for crop improvement. This paper describes the existing quarantine systems in India, highlighting main features of the Indian Quarantine Act and listing some of the pests and diseases intercepted on imported tree seeds. Information is provided on successful introductions of exotic tree species into India; conservation of genetic resources of tree species and future conservation activities have also been discussed.

EXCHANGE of seed/planting materials has helped in extending and diversifying agriculture and also in increasing crop production throughout the world. This has also led to introduction of new pathogens and pests in different countries from one part to another. In recent years, large scale exchange of plant genetic resources and rapid transportation of plant materials have been made through air freight/accompanied baggage. In practice, the international barriers are disappearing, and this has considerably increased the risk of introduction of new and serious plant diseases and pests from one country to another, as well as their spread within the country. There are numerous well documented instances of introduction of new diseases in places where they were not known earlier.

In view of the serious hazards anticipated due to frequent exchange of materials, it is imperative that each country has an efficient quarantine system to safeguard the possible entry of new pests and pathogens while exchanging germplasm (seed, planting materials) for crop improvement purposes.

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Import Regulations/Plant Quarantine

In India, import and export of seeds, plant products and planting materials are regulated by the rules and regulations framed under the Destructive Insects and Pests Act (DIP Act) of 1914 (extended by Directorate of Plant Protection, Quarantine and Storage, Ministry of Agriculture and Irrigation, 1976) which has been revised subsequently from time to time by the Government of India.

The main objective of this Act is to prevent the introduction into the country, and also the transport from one state to another, of any insect, fungus, or other pest, which is or may be destructive to crops. Seed was not originally included in the DIP Act but, in 1984, the Government of India passed the Plants, Fruits and Seeds (Regulation of Imports into India) order that came into effect in June 1985. This order is quite comprehensive, encompassing 17 crops and stipulating conditions for their import. Main features of this order are as follows:

- Seed has been brought under preview of the DIP Act.
- No seed/plant material can be imported into India without a valid Import Permit issued by the Plant Protection Advisor, Government of India, Ministry of Agriculture.

- No consignment can be imported into India without an official phytosanitary certificate issued by the plant quarantine agency of the exporting country.
- Post-entry isolation growing of specified crops at approved locations is stipulated.

Authorised Quarantine Agencies

The Directorate of Plant Protection, Quarantine and Storage under the Ministry of Agriculture, Government of India, implements the plant quarantine rules framed under the DIP Act. This organisation handles bulk import and export of seeds/plant materials for commercial purposes through its 26 plant quarantine and fumigation stations at 10 international airports, nine seaports and seven land frontiers.

The Government of India has also authorised three other national institutions for import, export and quarantine clearance of seed/plant material imported for research. These are:

1) National Bureau of Plant Genetic Resources (NBPGR) at New Delhi to import and export seeds/planting material of agri-horticultural and silvicultural crops for research purposes in respect of the institutes, projects and state agricultural universities in the Indian Council of Agricultural Research (ICAR) system.

2) The Forest Research Institute (FRI), Dehradun, for forestry plants (Department of Environment and Forests).

3) The Botanical Survey of India (BSI), Calcutta for plants of botanical interest (Department of Environment and Forests).

Germplasm Exchange

Systematic activities on exchange of plant genetic resources of agri-horticultural crops started as early as 1946 at the Indian Agricultural Research Institute (IARI), New Delhi, under a scheme initiated in its Division of Botany on the advice of ICAR (Pal and Singh 1949). In 1956, the scheme was replaced by the Plant Introduction and Exploration Organisation located in the same Division. In 1961, a new Division of Plant Introduction was created at the IARI, New Delhi.

In 1976, an independent institute, the National Bureau of Plant Genetic Resources (NBPGR) was established. The NBPGR has been functioning as a nation-wide research-cum-service organisation for exchange, assisting various All India Crop Improvement Programmes, ICAR Crop-based Institutes, state agricultural universities, forestry departments as well as agri-horticultural departments in the country. The Bureau has close linkages for germplasm exchange with more than 80 countries, as well as international institutes under the CGIAR system, viz; IRRI (Philippines), CIMMYT (Mexico), CIAT (Colombia), CIP (Peru), ICRISAT (India) as well as other centres like AVRDC (Taiwan) and WARDA (Liberia).

The Division of Germplasm Exchange has continued its efforts of introduction/exchange of germplasm of agri-horticultural and agri-silvicultural plants and, on average, 10,000–15,000 lots of germplasm material (seeds/planting materials) are introduced every year from different countries through correspondence. In addition, 30,000–40,000 accessions including segregating lines and trial entries are introduced from international institutes in crops like rice, wheat, barley, maize, pulses and tree species for multilocational testing under strict quarantine control.

ICAR has emphasised the need for integrated rural development, involving social-farm-agroforestry in order to promote agrarian prosperity and to bring continuing benefits to the native communities. In this context, the introduction of multipurpose trees has received wide interest and urgent attention. Consequently, activities related to the introduction of silvi-agricultural plants has tremendously increased during the last decade, with growing emphasis on introduction of materials for social forestry and farm forestry/range land management programs.

Procedure for Exchange of Germplasm

The NBPGR has brought out a brochure 'Guidelines for the exchange of seed/planting material' (National Bureau of Plant Genetic Resources, 1986) and it has been circulated widely among scientists in India. These guidelines are also applicable for the exchange of seed material of trees and shrubs.

Important Interceptions in Tree Species

Plant Quarantine Division of the Bureau has intercepted a number of insect pests, nematodes and pathogens in imported tree species seeds during the last ten years. Some of these are listed in Table 1.

Some Promising Introductions

The Bureau has introduced several silvi-agricultural plants with more emphasis on social forestry, farm forestry and range management. Germplasm of multipurpose trees has been introduced mainly from the Australian Tree Seed

Table 1. Interceptions of parasitic fungi, nematodes and pests on imported seeds and planting materials of fruit and tree species

Fungi intercepted	Crop species	Country of origin
Alternaria zinniae M.B. Ellis	*Carica papaya*	Brazil
Botrytis cinerea Pers, ex Pers	*Ficus* spp.	USA
" " " " "	*Vitis* spp.	Belgium, Czechoslovakia, Switzerland, USA, USSR
" " " " "	*Calligonum* spp.	USSR
Colletrotrichum dematicum (Pers. ex. Fr) Grove	*Leucaena leucocephala*	USA
" " " " "	*Leucaena* spp.	USA
" " " " "	*Pyrus* spp.	Malawi
Drechslera sorokiniana (Sacc.) Subram & Jain	*Atriplex* sp.	Australia
Fusarium culmorum (W.G. Smith) Sacc.	*Acacia* sp.	Australia
Fusarium oxysporum Schlecht, ex. Fr	*Leucaena* spp.	USA
" " "	*Simmondsia chinensis*	Africa
F. solani (Mart.) Sacc.	*Acacia* sp.	Australia
" " "	*Casuarina* spp.	Australia
Nematodes intercepted:		
Alaimus sp	*Macadamia integrifolia*	Australia
Alaimus sp	*M. tetraphylla* seeds (debris)	
Aphelenchoides basseyi	*Guiliema gasipae*	Brazil
Aphelenchoides spp	Plum, peach, cherry	Bulgaria
" "	*Prunus* spp (plants)	
" "	*Citrus bergamia*	Italy
" "	*Persica americana* (rooted/plants)	Australia
" "	*Acrocomia sclerocarpa*	Brazil
" "	*Enterpe oleracea*	Brazil
" "	*Macadamia intregrifolia*	Australia
" "	*M. tetraphylla*	Australia
" "	*Eucalyptus* spp (soilclods)	Australia
Buriaphelenchus sp	*Elaeis guineensis*	Indonesia
Pests intercepted:		
Alphitobius laevigatus (Fabricius)	*Elaeis guineensis*	Fiji
Amblyseicus sp	*Prunus* sp	Yugoslavia
Araecerus sp	*Leucaena* sp	Philippines
A. fasciculatus De Geer	*Leucaena diversifolia*	Philippines, Thailand
" " "	*L. leucocephala*	USA
A. levipennis Jordon	*L. leucocephala*	USA
Bruchidius sp	*Acacia albida*	Australia
Carpocapsa pomonella (Linnaeus)	*Juglans regia*	USSR
Cheylatus cruditus (Schrank)	*Olea* sp	USSR
Horismenus depressus Gahan	*Acacia* sp	USA
Ptinus exularis Erichson	*Eucalyptus obliqua*	Australia
Rhodocarpus sp	*Prunus avium* (cuttings)	Netherlands
Stator pruinins (Horn)	*Leucaena leucocephala*	USA
S. limbatus (Horn)	*Acacia* sp	USA
Stegobium sp	*Prosopis* sp	Argentina
* Bruchids	*Acacia albida A. podalyriifolia*	Australia
"	*Leucaena* spp	
* Hymenoptera	*Acacia* sp	Australia
* Lepidoptera	*Eucalyptus* sp	Kenya
"	*Castanea* sp	France
* Mites	*Bactris gassipaes*	Brazil
"	*Citrus* spp	Czechoslovakia
* Scales	*Citrus hodera*	Czechoslovakia

* identification in progress.

Centre, CSIRO, Division of Forestry and Forest Products, Canberra; Oxford Forestry Institute, Oxford, U.K.; Nitrogen Fixing Tree Association, Hawaii; National Arboretum, Washington D.C.; and also from France, Brazil and Philippines under strict phytosanitary conditions. Over 600 collections of trees/shrubs have been introduced during the last five years (1984–88) from over 20 source countries. Among these, the more important introductions included species of *Acacia, Albizia, Atriplex* spp., *Calliandra calothyrsus, Callitris* spp., *Ceratonia siliqua, Casuarina, Allocasuarina* spp., *Leucaena, Eucalyptus, Terminalia, Mimosa* spp., *Sesbania* spp., *Prosopis* spp., Pinus spp., *Cassia* spp., *Melaleuca* spp., *Pittosporum* spp., and *Gliricidia.* Major collections are of *Leucaena* (250) from Australia, USA, Philippines, Ethiopia, Syria, and U.K.; *Casuarina* (35) from Australia and USA; *Eucalyptus* spp. (85) from Australia, U.K., and Argentina; *Prosopis* spp. (45) from Denmark, U.K., USA, China and Argentina; *Robinia pseudoacacia* (30), USA and U.K.; *Haematoxylon brasiletta, Salix* spp. and *Populus maximoviczii* (ex Japan).

The research work carried out mainly at the Central Arid Zone Research Institute (CAZRI) and NBPGR Regional Station, Jodhpur, Indian Grassland and Fodder Research Institute, Jhansi, various soil conservation centres and state forest departments/state forest institutes has resulted in identification of some useful primary introductions for various situations. Some noteworthy introductions are: Israel babul (*Acacia tortilis*) — a multipurpose species, for fuel, fodder and resin, and as an excellent soil stabiliser; *Prosopis tamarugo* — an evergreen plant species from Chile, which can grow in soils with thick crust of salt and can become useful for year round fodder in salt affected region; *Colophospermum mopane* — a fodder tree species from southern Zimbabwe, suitable for extreme arid conditions; *Brosimum alicastrum* — a native of Southern Mexico, extremely tolerant to drought and excellent for forage and timber; *Acacia albida* — an introduction from Africa, an excellent species for agroforestry systems; *Cassia sturtii* — an introduction from Australia, with excellent potential to grow in sandy and slightly alkaline soils in low rainfall areas; *Brachychiton populneus* — a drought-tolerant shade tree of Australian origin, provides finest fodder and is also useful as a windbreak when planted in multiple rows; Kumut (*Acacia senegal* Wild) — an introduction from Africa provides best grade of gum arabic; *Atriplex halimus* (EC 129767 ex Tunisia), *A. canescens* (EC 129768 ex Tunisia) and *A. nummularia* (EC 129766 ex Tunisia) which are perennial, evergreen and salt-tolerant have performed well at Jodhpur and hold promise for arid regions.

Among the acacias *A. albida*, an introduction, holds great promise for arid/semi-arid habitats. Its pods and foliage provide good fodder. Ipil ipil (*Leucaena leucocephala*) — a fast growing multipurpose tree for forage, firewood and small timber assumes special significance in Indian agroforestry. The exotic introductions of *Leucaena leucocephala* like K. 8 (EC 124343 ex Philippines) and El Salvador (EC 123866 ex Australia) have proved superior for fodder and fuel in Gujarat and Kerala states.

Amongst the shrubs of industrial utility are jojoba (*Simmondsia chinensis*), a native of northern Mexico and southern USA valued for its seed oil or liquid wax for various industrial uses; guayule (*Parthenium argentatum*), a desert shrub from north central Mexico and southwestern USA, a drought-hardy plant having potential for rubber production; and an edible-oil-yielding tree, *Simarouba glauca* ex Salvador.

Although estimates of the extent of wastelands in India have varied a great deal for want of systematic surveys for this purpose about 158 million ha are considered to be severely degraded. Suitable tree species for rangeland improvement and wasteland development under different situations on problematic land have been identified (Singh 1988).

Seed Supplies for Overseas

In recent years, good amounts of seed material have been supplied by the Bureau to many countries, namely Nigeria, Bolivia, Switzerland, Niger, Dominica, USA, Brazil, USSR, Tanzania, Italy, West Germany, Tunisia and others. The trees genetic resources supply includes diverse material like *Acacia* spp., *Azadiracter indica* (neem), *Albizia lebbeck, Prosopis cineraria, Sesbania* spp., *Casuarina equisetifolia, Populus ciliata, Parkinsonia aculeata, Dalbergia sissoo* to mention some of the indigenous and exotics of agri-silvi-cultural importance.

Conservation

An important area for conservation activities is the establishment of gene sanctuaries, i.e., specific pockets of genetic diversity of crop plants and their related wild species which can be conserved in situ. One such gene sanctuary is proposed in the Garo Hills of the northeastern region for *Citrus indica* (Singh 1981). It is also envisaged to preserve *Musa, Mangifera, Citrus* species and other

economically important plants occurring naturally as wild/cultivated populations or as semiprotected populations. Similarly, there is a need to plan gene sanctuaries for important forest tree species as well.

Botanical Survey of India (BSI) has identified experimental gardens for forest trees gene sanctuaries: Gymnosperm gene sanctuary at Pauri in Uttar Pradesh; (ii) bamboo gene sanctuaries at Arunachal Pradesh and Kerala. It is also proposed to develop biosphere reserves in 29 endemic centres of the country so that conservation of endangered species can be best protected.

Emerging Thrust Areas

The following aspects are receiving attention for mobilisation of funding support:

(1) Germplasm collection of selected tree species of indigenous and promising exotic materials, particularly relevant to development of wastelands and regeneration of vegetation cover.

(2) Systematic maintenance, characterisation, evaluation and documentation of such collected germplasm.

(3) Development of efficient quarantine procedures for detection of various pests and pathogens as related to introduction of exotic materials.

(4) Conservation of native forest genetic resources under different distinct ecological situations.

More emphasis is required for the introduction of fast-growing multipurpose trees and shrubs for various purposes e.g., *Paulownia* (China's magic tree), *Salix* spp., *Populus* spp., *Pseudoacacia* and bamboo species from China; *Acacia senegal* provenances with high gum-yielding capabilities from Ethiopia, Nigeria and Senegal, and the introduction of selected germplasm collections in *Pinus* and *Eucalyptus* from CENARGEN/EMBRAPA, Brazil. Emphasis is also needed on introduction of multipurpose dry zone hardwood species from USA, U.K. and other sources like CSIRO, Australia (Turnbull 1986). There is also need for introduction of more germplasm in *Acacia albida, Simrouba glauca* and other useful tree species. Nitrogen fixing multipurpose trees suited to silviagricultural system will obviously receive greater attention.

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Improving the Forest Seed Situation in some African Countries

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Abstract

Serious deforestation has occurred in many African countries. To counteract this situation and to stop desertification, reforestation is urgently required. Several African countries are short of forest seed for reforestation programs. Local collection of seed is difficult and imports limited, and poor processing techniques often reduce germination capacity and storability. Seed is damaged through inadequate storage, as well as insects and diseases. Methods to germinate seeds of many indigenous species are not known, and very few of these are included in the International Seed Testing Association's Rules for Seed Testing. There is a shortage of trained personnel, equipment and funds in most African countries to carry out the seed work. Seed problems requiring research are identified and the training needs pointed out. Measures are suggested to improve the situation concerning forest seed.

FIREWOOD is the main source of energy throughout Africa. Since the production of firewood is far below the demand, there is a chronic energy crisis in many African countries. Due to shortage of land for cultivation in several countries in Africa, agriculture and forestry have started cooperating, leading to 'agroforestry'. In agroforestry systems, trees are planted on the farmland and serve not only as windbreaks, sources of shade, fire- and construction wood, but also other purposes (e.g. gum collection, fruit production, fodder for domestic animals, etc). Thus the trees form an integral part of the land use and are planted on the farmland, along the farm-roads, and elsewhere. Since the trees are meant for various uses, increased emphasis is being placed on 'multipurpose species'.

With the exception of those species which are propagated vegetatively, trees are raised from seed. Seeds give rise to trees and the trees in turn give rise to seeds. To produce vigorous, healthy trees, good quality seed has to be sown. However, obtaining adequate quantities of good quality seed, free from diseases and pests and with high purity and germination, is a serious problem in many African countries.

This paper reviews the situation concerning forest seed in some Sub-Sahelian countries in Africa, and suggests measures for improvement.

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Important Forestry Species

Table 1 lists some of the important indigenous and exotic forestry species in alphabetical order in some African countries.

Seed Collection

One of the main difficulties for seed collection in many African countries is the lack of information. Little is known about the flowering and fruiting habits and the maturity indices of fruits/seeds of the indigenous species. Although several tree species flower and fruit regularly, there are always some which do not. Some species flower and fruit profusely in certain years and sparsely in others. Apart from periodicity, there are species which produce limited quantities of seeds and are called 'shy' seeders. As a result of

Table 1. Important indigenous and exotic forestry species grown in eight African countries.

Country	Species
Botswana:	*Acacia* spp., *Afzelia quanzensis, Baikiaea plurijuga, Combretum* spp., *Entandrophragma caudatum, Eucalyptus camaldulensis, E. paniculata, Pterocarpus angolensis,* and *Terminalia* spp. (cf. Alidi 1985; Michaelsen and Millar 1987; Persson 1975).
Burkina Faso:	*Acacia albida, A. nilotica, A. raddiana, Azadirachta indica, Bauhinia rufescens, Eucalyptus camaldulensis, Gmelina arborea, Khaya senegalensis, Leucaena leucocephala, Parkia biglobosa, Parkinsonia aculeata, Prosopis juliflora, Ziziphus mauritiana,* etc. (cf. Ouedraogo 1988; Ouedraogo and Verwey 1987).
Ethiopia:	*Acacia saligna, A. decurrens, A. melanoxylon, Casuarina equisetifolia, Cordia africana, Cupressus lusitanica, Eucalyptus camaldulensis, E. globulus, E. saligna, E. tereticornis, Grevillea robusta, Hagenia abyssinica, Juniperus procera, Pinus patula, Podocarpus gracilior,* etc. (cf. Ibrahim 1985; Tadele 1987)
Kenya:	*Calliandra calothyrsus, Croton machrostachys, Cupressus lusitanica, Eucalyptus* spp., *Gliricidia sepium, Grevillea robusta, Leucaena leucocephala, Markhamia platycalyx, Mimosa scabrella, Parkinsonia aculeata, Pinus patula, Prosopis chilensis, Sesbania sesban,* etc. (cf. Mung-ala 1987, Rode 1987).
Mozambique:	*Afzelia quanzensis, Albizia versicolor, Amblygonocarpus andongensis, Burkea africana, Chlorophora excelsa, Dalbergia melanoxylon, Erythrophleum suaveolens, Eucalyptus camaldulensis, E. tereticornis, Khaya nyasica, Millettia stuhlmannii, Pinus caribaea, P. oocarpa,* and *Pterocarpus angolensis,* etc. (cf. Persson 1985; Rudin 1987; Wate 1987).
Nigeria:	*Afzelia africana, Azadirachta indica, Chlorophora excelsa, Gmelina arborea, Mitragya* spp., *Nauclea diderrichii, Pinus caribaea, P. oocarpa, Tectona grandis, Terminalia ivorensis, T. superba, Triplochiton scleroxylon,* etc. (cf. Okoro and Dada 1987; Persson 1975).
Somalia:	*Acacia hockii, Afzelia quanzensis, Azadirachta indica, Buxus hildebrandtii, Casuarina equisetifolia, Conocarpus lancifolius, Cordia ravae, Dodonaea viscosa, Eucalyptus* spp., *Juniperus procera, Leucaena* spp., *Olea africana, Prosopis juliflora, Sideroxylon buxifolium, Terminalia bispindosa,* etc. (cf. Abdisamed 1987, Persson 1975).
Zambia:	*Baikiaea plurijuga, Brachystegia* spp., *Eucalyptus camaldulensis, E. cloeziana, E. grandis, E. tereticornis, Khaya nyasica, Pinus kesiya, P. oocarpa, Pterocarpus angolensis,* etc. (cf. Shakacite 1987).

such factors, it is not always possible to plan collection operations effectively or to make optimal seed collection. Consequently, to obtaining sufficient quantities of seed at the suitable time for sowing is a serious problem in many African countries.

To save costs, or for other reasons, seed is often collected locally, wherever possible. Only when vehicles and funds are available can collections be undertaken at distant sites. With few exceptions, seed is collected by untrained labour, often unsupervised. Usually the collectors have practically no knowledge of scientific principles of seed collection. Often they collect seed from isolated trees which are profuse seeders and easy to climb. Frequently seed from inbreeding such trees is of poor genetic quality. Moreover, commonly no data are recorded about the collection site or the trees from which seed is collected.

Depending upon the species and other factors, the seed is collected from the forest floor, from felled or standing trees. However, to collect suitable amounts of good quality seed, trees must be selected and usually climbed. Tree climbing is carried out unaided, with the help of ropes usually designed on the principle of the tree bicycle, or by ladders etc. The fruits are picked by hand or knocked off by hooks, poles or rakes, or the fruit stalks cut by a parrot head pole pruner. Knives and sickles tied to long poles to reach the tree branches are also used. Sometimes heavily fruiting branches are cut down on to canvas spread under the tree.

In most African countries there are no seed orchards, or they exist only for a few species, usually exotic. In others, there are no registered seed trees or registered seed stands from which collections can be made. In some countries registered seed stands exist, but since the seed quantities needed are large, seed is also collected from natural stands or from plantations established for other purposes.

After collection, fruits/seeds have to be transported to a processing depot. During transport,

which is often slow in Africa, there is a high risk of damage to seed viability when temperature and relative humidity are high. Measures have therefore to be taken to avoid damage to seed (cf. Willan 1985).

Seed Processing

In most countries, seed extraction is carried out manually. It is a laborious and time-consuming operation. In several countries (e.g. Zambia, Tanzania) the cones of pines, the capsules of eucalypts and the pods of *Acacia* are dried in the sun on concrete floors, shallow wire mesh trays or on canvas and shaken. However, in some species the fruits/cones/capsules are dried in an oven at suitable temperatures and then opened by hand or flailed to release the seeds (Shakacite 1987). The pulpy fruits of *Azadirachta indica, Cinnamomum camphora* and *Maesopsis eminii* are macerated and the seeds are separated from the pulp by washing in water and then dried (Shehaghilo 1985).

In Nigeria seeds are divided into three categories for processing: (1) pods or capsules (e.g. *Afzelia africana*) which either split on the trees and release seeds, or are harvested green, then dried and split to release seeds; (2) dry one-seeded fruits (e.g. *Terminalia* spp. and *Triplochiton scleroxylon*) need to be dewinged manually, either by rubbing between the hands or by threshing in cloth bags and blowing off the chaff; (3) berries and drupes with fleshy pericarp which is removed by fermentation in heaps (*Gmelina arborea*), in pits (*Canarium schweinfurthii*), soaking overnight in water (*Chlorophora excelsa*), or depulping in fresh water (*Nauclea diderrichii*), or by abrasion with sand (*Azadirachta indica*). Germination of seed is often reduced if the seeds are allowed to ferment. This could be due to the heat generated during the process or to the leaching of inhibitors from the fermented pulp (Okoro and Dada 1987).

Seed suffers mechanical damage during processing due to lack of suitable equipment and techniques in several African countries. Mechanically damaged seed loses its viability more rapidly than undamaged seed during storage (cf. Kamra 1967a). Such seed should be stored for only a short time before use. Mechanical damage to seed can be reliably detected by x-ray radiography (Kamra 1967b).

Seed Testing

After processing, it is necessary to test the seed before it is stored. Testing a seed lot provides an accurate estimate of its capacity to produce healthy and vigorous plants suitable for field planting. However, suitable methods for testing tropical seeds are often not available. ISTA Rules for many tropical forestry species have not been worked out, although some progress has been made in this matter during recent years. Lack of knowledge about seed testing procedures therefore continues to be a problem in seed programs of many African countries.

The facilities for seed testing and research vary from one country to another in Africa. Thus for example, Somalia, some Sahelian and other countries do not have any suitable seed laboratories, while a few have well-equipped ones (e.g. Burkina Faso and Zimbabwe). Ethiopia, Kenya and Tanzania have also seed laboratories, although Tanzania has shortage of equipment and trained personnel.

In Ethiopia tests are conducted for purity, germination and moisture content of the seeds. Only a few experiments concerning dormancy and longevity of the seeds of the indigenous species have been performed in recent years. Seed dormancy is an important problem in several indigenous species.

In Zambia, seed samples are tested to determine viability, germination, number of seeds per kilogram and health status. For species showing poor rates of germination, tests are undertaken to find the suitable medium or presowing treatment.

Seeds of some tropical species germinate easily while those of others such as *Terminalia ivorensis* slowly and poorly, or require specific pretreatments (e.g. some *Acacia* spp.). Information about specific pretreatments for indigenous species is lacking or limited in several countries. Research work on suitable pretreatments to stimulate germination is needed on many important African species.

Seed Storage

Storage of forest seeds in those African countries with high ambient temperature and humidity is a difficult problem. Fluctuations in temperature and relative humidity can lead to reduction or loss of viability. In addition, under these conditions the effect of attack by fungi or bacteria on seeds can be severe. Insects are another source of hazard to seeds. Thus facilities for controlled temperature and humidity are needed so that seeds can survive during storage.

Unfortunately, suitable seed storage facilities do not exist in several African countries. Thus for example all seeds are stored at the ambient temperature and relative humidity in Somalia. Seeds

of several important species lose their viability rapidly under such conditions. Only seeds of a few species (e.g. some hard-coated and well-dried legumes, and *Zizyphus* sp. etc.) stand this storage (Abdisamed 1987). In Tanzania most of the seed is kept at room temperature in an open store. Those species which do not store well there are maintained in a dehumidified room at 30% r.h. and ambient temperature. Seeds of most species remain viable under these low-humidity conditions, except large seeds of some hardwoods. Cold storage between −3 and +3°C is used for conifer seeds (Shehaghilo 1985). In Ethiopia, seed is stored in well-ventilated chambers at room temperature.

A cold storage facility is available at the Forestry Research Institute of Nigeria. By storing at +5°C the viability of seeds of several, otherwise short-lived, species (e.g. *Entandrophragma angolense, Khaya* spp. *Triplochiton scleroxylon*) is prolonged. By drying seed of *Triplochiton scleroxylon* to 8–10% moisture content, it can be stored at ambient temperature in sealed containers with silica gel for 9 months, and at −18°C for 18 months or longer (Howland and Bowen 1977). Also at Kitwe, Zambia, there is a cold room where up to 15 tonnes of seed can be stored. The room can be expanded to accommodate additional cooling equipment to facilitate bulk storage (Shakacite 1987).

At the National Forest Seed Centre, Ouagadougou, Burkina Faso, a cold store exists where large quantities of seeds of various species are maintained at 4°C and 35% r.h. (Centre National de Semences Forestières 1987).

Storing seed under ambient conditions has the disadvantage that the fluctuations in temperature and relative humidity lead to the deterioration of viability in a relatively short time. The containers used for storing seed also contribute to this result. Depending upon the species, seed moisture content and other factors, paper bags, plastic bags, sacks, glass bottles, metal tins, etc may or may not be the best containers. Since suitable criteria for seed moisture content, temperature and relative humidity are not known for many tropical species, viability losses occur during storage. This is particularly the case with the recalcitrant seeds. Thus for example the microbiotic seeds of *Azadirachta indica, Butyrospermum paradoxum, Lannea microcarpa, Sclerocarya birrea* lose their viability significantly after only one month of storage (Ouedraogo and Verwey 1987). Experience from Nigeria shows that seedlots of *Azadirachta indica* lose most of their viability when stored at 6–7°C for 12 weeks.

During storage, insects, fungi, or bacteria can cause considerable losses of seed. As an example, the seeds of *Acacia* and *Balanites* are usually damaged by Bruchid larvae. The larvae are already present in the seeds when collected and therefore the application of insecticides or the manipulation of storage conditions have little effect (Milimo 1987). Similar reports describing the attack on fruits and seeds of indigenous leguminous trees by members of the *Bruchidae*, particularly the genus *Bruchidus*, are available from Zambia (Shakacite 1987). Consequently, there is a need to establish procedures for routine pest control prior to and during seed storage in the African countries.

Seed Certification

Seed certification, in the true sense, usually refers to the genetic quality (genetic composition) of the seed. However to judge the seed quality fully, it is also necessary to take into account the physical and physiological quality as well as the health status of the seed. Seed certification is intended to authenticate the origin and improve the genetic quality of the seed used for sowing purposes. At present, certification of forest seed is not carried out in most African countries.

Seed Imports

If enough seed of a desired species cannot be collected within a country, it has to be imported. The shortage of foreign exchange is one limiting factor in this connection. Moreover, the experience concerning imported seed is often not good, as pointed out by Ouedraogo (1988) from Burkina Faso, Shehaghilo (1985) from Tanzania, Abdisamed (1987) from Somalia, and others. To improve the situation, Willan (1988) has made some proposals.

Conclusions and Recommendations

From the situation described above, the following observations can be made concerning forest seed in most African countries:

(1) Due to increased tree planting activities for various purposes, the demand for seed has risen appreciably.

(2) There is a shortage of seed for sowing purposes of indigenous or exotic species or both. Local collection of seed is often difficult (see below) and seed imports limited due to restrictions on foreign exchange or other factors.

(3) The collection of seed of indigenous species may be difficult for the following reasons: (a) lack of knowledge of the flowering and fruiting habits of the species concerned; (b) shortage of suitable

equipment for seed collection purposes; (c) lack of trained personnel to carry out seed collection operations effectively; (d) insect damage to seeds/fruits which makes them unsuitable for collection; (e) lack of data about the origin of a seed, which makes it difficult to obtain seed of the same origin at a later date.

(4) The viability of the collected seed is frequently reduced during transport and storage before processing.

(5) Seed processing is carried out manually. The techniques used often cause mechanical damage to seed resulting in further loss of viability. Suitable equipment and efficient methods for seed processing are rare.

(6) Most seed is commonly stored under ambient conditions of temperature and relative humidity which leads to still further reduction in viability. Cold storage facilities are nonexistent in many countries and available in only a few.

(7) Methods for germinating seeds of many African indigenous species are not known. Very few of them are included in the ISTA Rules for Seed Testing.

(8) Techniques to pretreat seeds to stimulate their germination have not been worked out for many species.

(9) Seed dormancy is found in several indigenous species. Methods to break it are unknown in many cases.

(10) Research on problems of collection, processing, testing and storage of seed, particularly of the indigenous species, is limited.

(11) Equipment and funds for seed research are inadequate.

(12) There is a shortage of trained personnel to carry out research on seeds.

To improve the situation, the following suggestions are made:

Research (cf. also Kamra and Ayling 1987) (1) develop maturity indices of fruits/seeds to facilitate collection of mature seeds; (2) improve equipment and techniques of seed collection; (3) improve equipment and methods of seed processing to reduce damage to seed; (4) investigate and establish the optimum conditions for seed germination (light, temperature, moisture, etc.); (5) develop rapid methods for determining seed viability which can supplement or replace germination tests; (6) find causes of seed dormancy and methods to break it; (7) determine suitable methods for seed storage to maintain viability; (8) develop suitable pretreatments to stimulate seed germination, so that more plants can be obtained from the same amount of seed; (9) improve techniques for health testing of seeds to determine diseases and pests; (10) undertake studies to stimulate flower and fruit production in tropical species where needed.

Training To counteract the shortage of trained personnel in African countries, it is necessary to organize training courses for technicians and professionals in seed technology work.

International aid Equipment, funds and consultants are required to carry out the research and training programs.

Regarding points (4) and (6) above, it is noted that investigations to establish optimum environment for seed germination and to find methods to break dormancy is a long-term process. Therefore development of rapid methods for determining seed viability (5) has to be carried out simultaneously. The rapid viability tests can be conducted by x-ray radiography or biochemical techniques. The x-ray radiography has the advantage that it not only enables the testing of seed viability, but also allows the determination of the content of empty, insect-attacked, mechanically damaged and poorly-developed seeds in a sample at the same time and without extra effort. The method therefore gives a good estimate of the quality of a seed sample. This information is vital for judging the suitability of a sample for raising seedlings and for calculating the sowing rate in the nursery. Consequently, the x-ray method is superior to the biochemical techniques and is therefore well suited to research on tropical seeds.

As a result of technical development over the years, the x-ray equipment today is neither particularly expensive nor does it require highly trained personnel. Inexpensive x-ray units are available and the personnel can be trained in a short time. Therefore the x-ray method is ideal for use in developing countries (cf. Kamra 1976). Regional Forest Seed Centres at Harare in Zimbabwe and Ouagadougou in Burkina Faso have already acquired x-ray machines for seed radiography work. Also seed centres in some other African countries (e.g. Ethiopia, Niger) are interested in purchasing x-ray machines for their work. Thus the potential of seed radiography in tropical forestry is being realised and the method is finding increased use in many countries, both in Africa and elsewhere.

The majority of African countries lack well-trained personnel, equipment and funds, and are in great need of help from more developed countries to carry out research work on their seed problems.

In conclusion, the following measures are necessary to solve the seed problems of Africa: (1) training of personnel at the technical and professional levels; (2) provision of equipment and funds for seed collection and processing; (3) use of consultants for short or long periods to guide research and other seed work. These measures can help to improve the situation and make it possible

to provide seed of good quality for afforestation and reforestation programs in Africa. These programs in turn can help counteract the problem of desertification facing the Sahelian and several other African countries.

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Seed Testing Research on Species Indigenous to Kenya

C. Schaefer*

Abstract

The objective of the Kenya Forestry Seed Centre (KFSC), in existence since 1985, is to provide sufficient quantities of seed for the afforestation programs of the country, with emphasis on indigenous species. Research at KFSC concentrates on suitable germination pretreatment techniques that are practicable in the field and also storability of these species about which little is known. To gather information quickly, it seems more efficient to submit fresh seeds of the priority species to comprehensive tests schematically. Such schemes are suggested for: (a) pretreatments of hard-coated seeds; (b) tests of drying and storing of seeds with a high initial moisture content, eventually recalcitrant seeds: (c) tests of the effect of the pulp on germination capacity of the seed. Results of germination and storage experiments of *Dobera glabra, Podocarpus milanjianus, Polyscias kikuyuensis, Prunus africana, Vitex keniensis* are presented.

The Kenya Forestry Seed Centre

THE Kenya Forestry Seed Centre (KFSC) was established in 1985. It is part of the Kenya Forestry Research Institute (KEFRI) at Muguga, some 25 km northwest of Nairobi. A smaller seed supply unit had existed within the Silviculture Division before 1985, dealing mainly with exotic softwood plantation species of *Pinus patula, P. radiata, P. caribaea, Cupressus lusitanica,* and *Eucalyptus* spp., as well as a few ornamental trees. The establishment of the Seed Centre was funded by the West German government.

Objectives of KFSC

There were two main objectives set by the Kenya government for the Seed Centre:

i) Increase in the quantity of seeds distributed to all kinds of nurseries in the country. The government has set the target of raising and planting 200 million tree seedlings yearly in order to cope with the sharply increasing demand for wood products, protect the environment, ensure the water supply, and rehabilitate degraded semi-arid lands.

ii) Emphasis on indigenous species. Very few indigenous tree species were planted to any extent worth mentioning until a few years ago. Thus, promoting the use of indigenous species required:
   — identification of appropriate seed sources
   — organisation of regular collection from the sources
   — procurement of information about extraction, proper drying and storage, germination pretreatments (i.e. practically-oriented research and dissemination of findings). That means working out methods for a technical level applicable in field nurseries of a developing country.

iii) A third objective developed by the project is the quality of the dispatched seedlots:
   — physiological quality is reached by proper timing of collection, and appropriate drying, storage, and transport measures
   — genetic quality is achieved by safeguarding genetic diversity of the germplasm collected, keeping different provenances separately, and, in a few cases of important timber species, by

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selecting a sufficient number of seed trees for their superior phenological quality.

Development of KFSC

In the few years of its existence, the Seed Centre has continuously increased the quantities of seeds harvested, dispatched, stored, as well as the species handled (Fig. 1). Whereas the latter figure will probably not rise considerably any more, demand and consequently collection and dispatch will continue to go up with increasing popularity of the Seed Centre within the country and abroad.

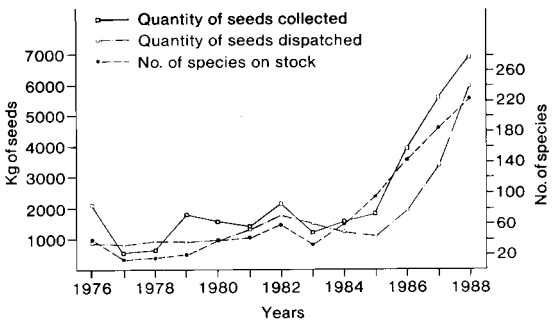

Fig. 1. Kenya Forestry Seed Centre activities, 1976–88.

Organisation and Equipment

The headquarters of KFSC is supported by six collection centres in different parts and ecological zones of the country. Two more outstations on a somewhat lower level are proposed to cover a large part of the arid and semi-arid lands (ASALs).

Seeds are stored in the headquarters' cold store at +3°C, which in case of power failures is backed up by standby diesel generator. For gene conservation purposes, the deep freeze chambers (−18°C) of the Gene Bank of Kenya are used.

Seed testing is carried out in parallel tests in the lab (germination tank or germination cabinet) and out in the open—highland species in the nursery, lowland and coastal species in the glasshouse. Laboratory results indicate theoretical germination chances, but outdoor results reflect germination capacities that can be achieved out in the field and are thus often more important in routine testing and practically-oriented research work.

Research on Seeds of Indigenous Species

The majority of the seeds of indigenous species have never been handled scientifically either in Kenya or abroad. A list of priority species (Table 1) based on the assumed importance for future large-scale plantations as well as social forestry serves as a guideline, showing which species to test out of almost 2000 woody plant species, approximately 200 of which are of higher importance.

The first indications on how to handle the seeds are given by the seed structure and the moisture content of the mature, freshly harvested and extracted seeds. The easiest, since they are the most predictable in their reactions, are the hard-coated seeds harvested at a moisture content (MC) of less than 15%. The most delicate seeds are pulpy fruits and, in general, seeds with a high MC of more than 45% at the time of maturity. The viability of the seedlot is estimated at the beginning of any experiment by a cutting test and the moisture content is determined.

Germination Pretreatment of Hard-coated Seeds

Seeds of the Leguminosae genera (*Acacia, Albizia, Dichrostachys, Entada* (Mimosoideae), *Erythrina, Sesbania* (Papilionoideae), *Bauhinia, Brachystegia, Caesalpinia, Cassia, Delonix, Erythrophleum, Paramacrobium, Piliostigma, Tamarindus, Trachylobium,* (Caesalpinioideae) (Taxonomy of Royal Botanic Gardens, and Dale and Greenway 1961, used) occurring in Kenya have a rather hard seedcoat and a low MC when mature. Storage is no problem, even for longer periods provided seeds are well dried to a MC of 4–8% and stored in an airtight container in the cold store (Willan 1985).

Hardcoatedness of the seed normally forms a barrier to water uptake or radicle emergence (Ellis et al. 1985). Therefore, certain presowing treatment is required to break this so-called seedcoat dormancy (Turnbull 1975). Kemp (1975) enumerates seed scarification, soaking in water, chemical treatment, stratification and radioactive and sound treatments. Willan (1985) additionally mentions biological methods, and dry heat and fire as appropriate measures. Stratification has proved to be suitable to break embryo dormancy of temperate zone seeds, but seems to be less appropriate for hard-coated tropical seeds. However, the sophisticated chemical and biological methods as well as radioactive and sound treatments contradict the aim of a practically-oriented research.

Comprehensive reports about successful pretreatments are found in Doran et al. (1983) for *Acacia* species, others are mentioned in National Academy of Sciences (1980, 1983) about various firewood crops and in Willan (1985). Kariuki (1987) reports successful pretreatments of *Acacia xanthophloea* and *Trachylobium,* two indigenous species of Kenya. Wanyondu (1989) found ways

Table 1. List of seed research priorities species, Kenya Forestry Seed Centre, July 1989.

Species	Field of necessary research			
	Extraction	Drying	Germination pretreatment	Storage
Aningeria adolfi-friederici				x
Balanites aegyptiaca		x	x	x
Boscia coriacea		x	x	x
Brachylaena hutchinsii		x		x
Brachystegia spiciformis		x	x	
Celtis africana	x	x	x	x
Chlorophora excelsa	x	x		x
Combretum molle or schumanii		x	x	x
Cordia abyssinica	x	x	x	x
C. sinensis		x	x	x
Croton macrostachys		x		x
Dalbergia melanoxylon		x	x	x
Diospyros abyssinica		x	x	x
D. mespiliformis	x			x
Dobera glabra		x		x
Dombeya goetzeni		x	x	
Fagara macrophylla				
Fagaropsis angolensis	x			x
Hagenia abyssinica	x	x	x	x
Juniperus procera		x	x	x
Maesopsis eminii	x	x	x	x
Melia volkensii		x	x	
Newtonia buchananii		x		x
Ocotea usambarensis		x		x
Olea africana	x	x	x	x
O. hochstetteri	x	x	x	x
O. welwitschii	x	x	x	x
Podocarpus gracilior		x		x
Premna maxima	x	x	x	x
Salvadora persica		x		x
Terminalia brownii				x
T. prunoides		x	x	x
Trichilia emetica		x		x
Vepris glandulosa	x	x	x	x
Vitex doniana	x	x	x	x
Warburgia ugandensis		x		x
Ziziphus mauritiana	x		x	

N.B. The list comprises species of highest importance with severe germination or storage problems and/or entire lack of information for handling, storage, and germination pretreatment.

to improve germination of *Sesbania sesban* and *S. grandiflora.*

Since it is hard to predict which of the different pretreatments will be the most successful, it is advisable to screen a species in a system of pretreatment tests (Table 2) to achieve the highest and quickest germination as economically as possible. A comprehensive experiment to tackle a hard-coated seed new to seed science could be as follows:

1. no pretreatment (control)
2. soaking in room temperature water (24 hours, 48 hours)
3. soaking in hot water (tests with water initially at 80°C, 90°C, boiling) and allow to cool overnight
4. (a) immersion in hot water (tests with water initially at 80°C, 90°C, and immersion times of 30 sec, 1, 2, 3, 5, 10 min), eventually subsequent soaking in room temperature water overnight
   (b) immersion in water kept boiling during immersion (10, 30 sec, 1, 2, 3, 5 min), eventually subsequent soaking in room temperature water overnight
5. soaking in conc. H_2SO_4 (tests lasting 1, 3, 5, 10, 15, 20, 30, 45, 60 min)
6. mechanical scarification (nipping at micropylar end, hot wire, sand paper, DANIDA 'seed gun') eventually followed by soaking in room temperature water overnight

Table 2. Systematic pretreatment experiment of seeds with hard seedcoat or pericarp.

1. No treatment (control)
2. Soaking in room temperature water for 24 or 48 hr
3. Soaking in water heated to 80, 90, 95 or 100°C, and allowed to cool overnight
4. Immersion in hot water and allow to soak for a certain time
   a) seeds immersed in 80°C water and soaked for 1, 2, 3, 5 or 10 min
   b) seeds immersed in 90°C water and soaked for 30 sec, 1, 2, 3, 5 or 10 min
   c) seeds immersed in 95°C water and soaked for 30 sec, 1, 2, 3, 5 or 10 min
   d) seeds immersed in boiling water and soaked for 30 sec, 1, 2, 3, 5 or 10 min. Immersion may be followed by soaking in room temperature water overnight.
5. Immersion in water kept boiling during immersion time of 10 sec, 30 sec, 1, 2, 3 or 5 min. Immersion may be followed by soaking in room temperature water overnight.
6. Soaking in conc. H_2SO_4 for 1, 3, 5, 10, 15, 20, 30, 45 or 60 min
7. Mechanical scarification with/by
   a) hot wire,
   b) sandpaper,
   c) wire brush,
   d) nipping with knife, nail clipper, pliers
   e) DANIDA 'seed gun'
   f) legume scarification drum machine (used in agriculture)

   eventually followed by soaking in room temperature water overnight.
8. Oven heating
   a) heating temperature 90° for 5, 10 or 20 min
   b) heating temperature 95° for 5, 10 or 20 min
   c) heating temperature 100° for 5, 10 or 20 min
9. Fire scorching with dry grass
   Weight of grass twice or three times the seeds' weight

7. heating in the oven at 90°C, 95°C, or 100°C for 5, 10 or 20 minutes
8. fire scorching with a certain quantity of dry grass (weight equal to two, or three times the weight of the seeds).

The latter method has been tried successfully on *Erythrina abyssinica* and *Juniperus procera* by Laurent and Chamshama (1987) in Tanzania.

In a few cases of extremely thick and hard seedcoats longer treatments are required: e.g. *Acacia sieberiana*, for which 60 min immersion in water kept boiling was reported the most successful pretreatment (Willan 1985).

Mechanical scarification of the pericarp enhanced germination of non-leguminous species with hard seed covering structures tested at KFSC: *Terminalia brownii* seeds germinated at 55% if the pericarp, which apparently allows the seed to imbibe water, but forms a mechanical resistance to radicle growth, is cut open in a V-shaped way at the radicle end of the seed, compared with 8% if the seeds were left untreated. *Terminalia spinosa* also improved from 60% to 81% with the same pretreatment. Cracking the pericarp of *Ziziphus abyssinica* with a hammer resulted in a germination of 57% versus 13% (untreated).

The list of potentially successful pretreatments is extremely long and 50 different methods can easily be tested. However, only one major experiment per species is required and it is worth working out the best germination pretreatment method to be able to use seeds most economically. On the other hand, certain differences may occur from year to year among provenances and individuals largely due to variations of the thickness of the seedcoat within the same species.

A few pretreatments out of the list are only applicable in the laboratory, for example soaking in H_2SO_4, oven heating, and, to some extent, hot water pretreatments below boiling temperature, and hot wire scarification. Considering the Seed Centre's primarily practical research orientation, pretreatment tests with chemicals are carried out only to a smaller extent with H_2SO_4 in order to complete the test scientifically. But sulphuric acid pretreatment is not recommended to the field nurseries because of dangerous and uncertain handling, unavailability, and costs. However, it could be considered to despatch hard-coated seeds only after the best pretreatment possible has been applied, the seeds being re-dried if required. Experiments reported by Willan (1985) and Lauridsen and Stubsgaard (1987) have shown no decrease in the germination capacity of pretreated seeds for a sufficient time until sowing. With such a distribution system, it would be possible to apply chemical or other pretreatments that are not practicable in the field at the Seed Centre, if those methods prove to produce the best results.

Some of the practicability problems for pretreatment in field nurseries can be overcome by using low-tech methods. The sophisticated electrically-heated hot wire, for example, can perhaps be replaced by a fire-heated piece of metal.

Germination and Storage Tests of Species with High MC

Most mature tropical seeds with an MC of more than 40% are ready to germinate immediately after being shed. Their viability often lasts only as long as they retain a high MC. Loss of viability with decreasing MC indicates recalcitrance of the seeds. Thus seeds with such a high MC at maturity can be checked for their recalcitrance according to

Table 3. Test of recalcitrance of seeds with high initial MC (> 40%).

1. Germination test of fresh seeds.
2. Drying and germination tests of seeds at 40, 35, 30, 25, 15, 10, or 8 per cent MC.

the following pattern (Table 3), if entirely fresh seeds can be obtained:

1. direct sowing of fresh seeds
2. drying down to 40, 35, 30, 25, 15, 10, 8% MC subsequently and sowing at each mentioned level (Bonner, 1988, pers. comm.). Drying should be done only in a thin layer which is well ventilated and under shade as long as the MC exceeds 20%.

The second step in testing seeds with high MC is the test of storability (Table 4). During the above mentioned process of drying, samples large enough to be re-tested several times are taken and stored at each level of drying.

Experiments with temperate species (e.g. *Quercus robur*, Suszka and Tylkowski 1980) have shown that it is often advisable to mix the seeds with a storage medium, if the MC of the seeds needs to be maintained at more than 35%. The media help keep the MC at a high level and suppress growth of fungi under cold storage conditions. Thus it is suggested that samples be stored with an MC of 35% and above in different media such as damp sand, damp sawdust, or damp peat and compare them with storage without medium (control). The volume of the medium should be about twice that of the sample.

Experiments at KFSC proved that, contrary to what was stated in literature (Lamprecht 1986),

Table 4. Test of storability of seeds with high initial MC.

The test is carried out after a recalcitrance test. It should be tried to store seeds in fresh condition and at the lowest MC level at which the recalcitrance test produced good results.

1. Seeds of 45, 40, 35% MC are stored in:
   a) open blastic box or drum
   b) perforated polythene bag
   c) open plastic box (or perforated polythene bag) seeds mixed with damp sawdust (twice the seeds volume)
   d) open plastic box (or perforated polythene bag), seeds mixed with damp peat (twice the seeds volume)
   e) open plastic box (or perforated polythene bag), seeds mixed with damp sand (twice the seeds volume)

   NB Cold storage experiments only
   With lower MC storage media should be slightly drier
2. Seeds of 30, 25, 20% MC are stored in:
   a) thinner polythene bag
   b) airtight drum, lid to be lifted from time to time to allow gaseous exchange
3. Seeds of < 20% MC are stored in airtight containers in
   a) coldstore
   b) room temperature

   Germination tests are carried out after 1, 2, or 3 months depending on the delicacy of the seed and are then repeated every 2 or 3 months.

Comment for Tables 5–8

Germination capacity values within a group having different letters are significantly different at 0.05 level (ANOVA and LSD test, Sachs 1984).

seeds of *Podocarpus milanjianus* can be stored successfully under favourable conditions for at least a year: The most important factors are to maintain a high MC and to keep temperature at 1–4°C. Storage in sawdust produced the best results, allowing no drop in germination capacity over 12 months (Table 5). In another storage experiment, it could be demonstrated that seeds of *Prunus africana*, which are extremely sensitive to loss of MC, can be stored in the coldstore with the same success. The seedlot had been dried after extraction by mistake, but storage in different

Table 5. Storage and germination test on *Podocarpus milanjianus* (perforated polythene bag; MC of sawdust 37%, of peat 11%; coldstore +3°C; germination tests in glasshouse, sand, duration 90 days).

Storage time	Storage medium	MC %	Germination capacity %
before storage, fresh	—	43	69
3 months	control	42	64
	sawdust	48	63
	peat	43	73
9 months	control	42	51 a
	sawdust	53	69 c
	peat	39	60 b
12 months	control	45	50 a
	sawdust	58	72 b
	peat	38	50 a

Table 6. Storage and germination experiment on *Prunus africana*, depulped (open plastic box, coldstore +3°C; germination test in glasshouse, sand duration 75 days).

Storage time	Storage medium	MC %	Germination capacity %
Before storage after extraction and shade drying	—	15	4
2 months	control	18	3 a
	sand	48	13 b
	sawdust	48	33 c
	peat	48	35 c
5 months	control	18	8 a
	sawdust	50	62 b
	peat	50	74 b

media enabled the seeds to take up moisture again up to the original MC, and regain germination capacity (Table 6).

The lower the MC of the seeds, the drier the storage medium can be initially. The type of storage container has to be considered as well. Because of more intensive respiration at high MCs the container should allow gaseous exchange (Willan 1985), so that perforated plastic bags or open-topped or perforated rigid plastic containers should be used. At MCs lower than 30% sealed containers are better.

Storage of seeds with high MC seems to be reasonable only in cold stores run at 1–4°C; otherwise seeds start germinating and/or mould fungi begin to grow more or less rapidly. The high RH of approximately 95% in a cold store does not allow the storage media to dry out, so no further moistening is required.

A variant with seeds fumigated with a fungicide before storage should be included in the experimental setup since even at +3°C slow mould growth was observed. The experiments at KFSC show that damp sawdust seems to maintain (and even increase) seed MC best and suppresses growth of mould well. On the other hand, it apparently supports slight radicle growth, which however, is not necessarily adverse if seeds are sown without delay.

The stored seeds should be retested frequently to determine the actual germination capacity. At KFSC, the first retest after storage is carried out after 1, 2 or 3 months depending on the assumed delicacy of the seeds. Further tests follow every two or three months.

Experiments with Pulpy Fruits

Fleshy fruits should be depulped as soon as possible to avoid fermentation and heating (Willan 1985) or because the fruit may contain chemicals inhibiting germination (Ellis et al. 1985). However, field personnel often claim that

Table 7. Systematic test of pulp fruits (effect of pulp, recalcitrance test, storability test)

1. Germination test when fresh
   a) with pulp
   b) depulped
2. Drying to levels 2 to 8 (if applicable) of recalcitrance test and germination tests
   a) seeds with pulp
   b) seeds depulped freshly
   c) seeds dried with pulp and depulped just before germination test (if pulp still removable)
3. Storage as in storability test
   a) ≥25% MC freshly depulped seeds only
   b) ≤ 20% MC
      ba) freshly depulped seeds
      bb) seeds with pulp

   Germination test to be carried out after 1, 2, or 3 months depending on assumed delicacy of seed and then to be repeated every 2 or 3 months.

   A variant
      bc) seeds dried with pulp and depulped just before germination test

   should be included.

   The lowest MC that gives still satisfactory germination in recalcitrance test should be tried. If fresh seeds exceed an MC of 40%, storage at this level should also be attempted.

In case of species with hard pericarp, combinations of fresh seeds germination, recalcitrance and storability test with pretreatments might be necessary at all MC levels with depulped seeds and at ≤20% MC with pulped seeds, too.

it makes no difference whether fruits are sown with or without the pulp. Species like *Cordia* spp., *Olea* spp. with a thin pulp are often collected at a stage after the pulp has dried on the pericarp and can hardly be removed. So the effect of the pulp has to be closely observed in order to determine the importance of proper timing of harvest.

An experiment, which mostly combines testing the effect of the pulp on germination and storage test of seeds with high MC, is designed as shown in Table 7.

Experiments at KFSC with *Polyscias kikuyuensis* and *Dobera glabra* (Table 8) demonstrate the difference in germination capacity between depulped seeds and fresh seeds with the pulp retained: depulped seeds germinate with a significantly higher rate than seeds with pulp on. After a certain storage time the gap between seeds stored depulped and those left with pulp widens as shown in an experiment with *Prunus africana* (Table 8).

Species with a sticky pulp, like *Vitex* spp. do not allow the pulp to be removed when fresh. In this case, fruits are dried first, before the pulp can be macerated. Again, seeds of *Vitex keniensis* show the same germination pattern (Table 8) of enhanced germination of depulped seeds before and up to 6 months after storage.

There is an indication of a rapid drop in viability of some species when fruits are overmature. Brown and yellow/greenish fruits of *Warburgia ugandensis* were collected and the seeds sown separately. The seeds of the brown fruits yielded a germination rate of only 27% whereas 48% of the seeds from yellow/greenish fruits germinated. Nagaveni et al. (1987) report the same observation for fruits of *Azadirachta indica*.

Table 8. Germination tests on various species at KFSC.

A. Germination test on *Polyscias kikuyuensis* (only sunken seeds used after floating in water; germination test in lab, petri dishes, germination chamber 20°/30° in 12 hrs turn, duration 90 days).

Treatment	MC %	Germination capacity %
With pulp, store-dried for 5 days	12	1 a
Depulped, store-dried for 5 days	8	88 d
Store-dried for 20 days, then depulped		50b
With pulp, store-dried for 25 days, sun-dried for 2 days	5.2	1 a
Depulped, then store-dried for 25 days, sun-dried for 2 days	4.5	75c

B. Germination test on *Dobera glabra* (glasshouse, sand, duration 90 days).

Treatment	MC %	Germination capacity %
Fresh, with full pulp	50	8 a
Fresh, outer (green) pulp removed inner (red) pulp left on	51	57 b
Fresh, totally depulped	48	70 b

C. Germination test on *Prunus africana*, stored for 5 months (damp sand in open box, coldstore +3°C; glasshouse, sand, duration 75 days).

Pulp status	MC %	Germination capacity %
Stored with pulp	62	0
Stored depulped	50	39

D. Germination and storage test on *Vitex keniensis* (nursery, sand seedbeds, duration 90 days).

Storage time	Treatment	MC %	Germination capacity %
Before storage	fresh, with pulp	43	23 ab
	fresh, depulped	31	46 c
Before storage	dried 1 week, with pulp		16 a
	dried 1 week, depulped		49 c
	dried 3 weeks, with pulp		28 b
	dried 3 weeks, depulped		55 c
3 months storage	dried 1 week, with pulp	26	16 a
	dried 1 week, depulped	9.2	51 b
	dried 3 weeks, with pulp	11	14 a
	dried 3 weeks, depulped	8.4	50 b
6 months storage	dried 1 week, with pulp	27	9 a
	dried 1 week, depulped	12	52 c
	dried 3 weeks, with pulp	11	11 a
	dried 3 weeks, depulped	8.8	34 b

Conclusions

There are two major conclusions which appear to have general application:

- immediate depulping of fleshy fruits gives significantly better germination results;
- some seeds which must maintain a high moisture content can be stored for considerable time in the cold store, preferably mixed with a storage medium like damp sawdust or peat.

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Germination Problems of some Multipurpose Indigenous Tree Seeds in Tanzania

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Abstract

This paper describes the problems encountered in the germination of multipurpose indigenous tree seeds in Tanzania, a field which has been overlooked in the past. Seeds of indigenous multipurpose tree species which appear to have germination problems due to physical or physiological dormancy are listed. Present research being carried out and suggestions for future work are indicated.

In Tanzania, afforestation programs have been underway for some time, but these were mainly exotics on industrial plantations. The indigenous species were abundant and were used by inhabitants for their varied products including timber, firewood, fodder, fruits, ceremonial, medicines etc. Some tree species are now listed as endangered. Recently a need has emerged to plant indigenous multipurpose species. Due to population pressure and the cessation of shifting cultivation, people have to produce everything on their small areas of land, i.e. trees, crops and livestock, hence the need for agroforestry. For example, in the West Usambaras of Tanzania there is a great land shortage problem with a population density of about 100 persons/km^2. People are obliged to diversify their lands (shambas). Shifts to nonconventional lands require transfer of species, in which case good germinable seed is needed. By 1984 for example, the seed centre at Lushoto was distributing an annual average of 1500 kg of seed, comprising 200 indigenous and exotic species (Msanga and Shehaghilo 1984). In the first six months of 1989 the centre has already distributed 2000 kg of tree seed. This is only about 20% of total requirement because the centre is incapable of supplying the total demand. The increased agroforestry activity recently and the depletion of once abundant indigenous species, and the planting of more indigenous trees by the forestry sector has brought about an increased seed demand.

Documentation on germination of most exotics is readily available, but almost nonexistent for the indigenous species. Nursery observations over the last few years indicate that some work must be done to improve germination on the species listed in Table 1. This paper describes the problems encountered on the germination of multipurpose indigenous seeds in Tanzania.

Table 1. Species posing germination problems and their expected dormancy (physical or physiological).

Species	Dormancy
Acacia albida	Physical
Acacia tortilis	Physical
Albizia schimperana	Physiological
Borassus aethiopicum	Physical
Catha edulis	Physiological
Cordia africana	Physical
Clutia abyssinica	Physiological
Kigelia africana	Physical
Melia volkensii	Physical and physiological
Maesopsis eminii	Physiological
Sclerocarya cafra	Physical
Syzigium guineense	Physiological
Trema guineense	Physiological
Trichilia emetica	Physiological
Warburgia ugandensis	Physical and physiological
Vangueria infausta	Physical and physiological
Zizyphus mauritiana	Physical

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Problems of Germination

The germination of many indigenous tree seeds appears to be low in many cases; the reasons for this are not fully understood. Little work on germination of indigenous species was done in the past, because these species were naturally abundant and no major afforestation programs were undertaken. Some scattered information can be found in different nursery books and files but often basic information such as the time for seed to germinate, pretreatments and germination energy is not available.

Research work being carried out at the nurseries using different soil mixtures, sowing times, presowing treatments is not adequately planned and followed up. The laboratory facilities at the Forest Research Centre have only two ovens, four Jacobsen tanks and a weighing balance. Seed activities are frequently hampered by cuts in electricity. Lack of trained personnel to carry out proper and continuous research on seed is another impediment. For example, the few experiments carried out in 1985–87 were part of studies for a Masters degree at the university and not a research program of the centre.

Table 1 lists the species which have been found to have some germination problems if untreated. Most of the dormancy information on the listed species results from observations noted, file and nursery records, personal communications and the many questions asked by various people on how to successfully germinate these tree seeds when the traditional methods do not appear to work well. The physical appearance of the actual seed and the recent routine tests carried out by the author of this paper have helped to identify the problem areas which need to be studied in detail.

Germination may be affected by many factors from collection, processing and storage depending on species (USDA 1974) but essentially what is required for good germination is to break the dormancy, which may be physical or physiological. Some species appear to possess both. Msanga and Maghembe (1986), for example, have found *Albizia schimperana* seed possesses both physical and physiological dormancy. Other species such as *Maesopsis eminii* seed which has sporadic and prolonged germination appear to have mainly physiological dormancy (Mugasha and Msanga 1987). More studies are needed to improve the germination by increasing the germinability and shortening the dormancy period.

Future Work

Experiments have started with a few species like *Albizia schimperana, Catha edulis, Maesopsis eminii, Melia volkensii, Trema guineense, Trichilia emetica, Waburgia ugandensis* and *Vangueria infausta*, and results are not yet available. It is intended to lay out experiments so as to define the best and most economical methods for each species. These will be enhanced by phenological observations and studies of collection times, extraction methods and storage conditions which may affect germination.

Tanzania has many trees with characteristics which indicate they could make excellent multipurpose trees when grown in association with agriculture, e.g. *Rauvolfia caffra*, for medicines, firewood and domestic utensils is also a good coffee shade tree, *Crotolaria brandicteata* a useful leguminous shrub, and *Allanblackia stuhlmanii, Bridelia micrantha, Markhamia platycalyx, Brucea antidysenterica, Croton* spp., *Erythrina* spp., *Uapaca* spp., *Voacanga* spp. all have potential in agroforestry. Many of the species have received little attention previously so that there are no established germination procedures.

National and international cooperation is necessary. A planned Danish International Development Assistance (DANIDA) seed project will deal with seed production to meet industrial and nonindustrial tree seed requirements of both indigenous and exotics, but not research on problematic indigenous species.

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Seed Research on *Tectona grandis* in India

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Abstract

A review of the research work done in India on various aspects of the seeds of teak (*Tectona grandis* Linn. f. — family Verbenaceae) has been given on the basis of all traceable literature. It is apparent that there has been a clearcut progressive trend from the old days to the modern age, in the methodology used. There have been four main aspects which were investigated during the last 90 years. They are: (1) presowing treatments to the seeds; (2) provenance trials; (3) emptiness of fruits; (4) seed requirements for plantation programs.

TEAK (*Tectona grandis* Linn. f.) one of the most useful tree species, has been grown in India since the remote past. However, it is also a native of the tropical deciduous forests of the Indian subcontinent and thrives best in the natural edapho-climatic conditions in several parts of the country. It is virtually the most important tree species in tropical forestry and is extensively planted both as a native and as an exotic. Most research has concentrated on increasing its overall productivity.

Presowing Treatments

Investigations on teak seed started in India at the onset of the twentieth century when Hodgson (1900) attempted to enhance the germination of teak seeds by certain pretreatments. However, Bourke (1914) concluded from observations made during 1912–13 that teak seeds germinate better even without any pretreatment, after storing for one or two years, than do the freshly collected seeds.

Tuggerse (1925a) described and evaluated a number of methods that were believed to be useful for getting quick germination of teak seeds in large numbers. One such method is to scorch the seeds in a light running fire of dry leaves or grass, suggested probably by the idea that in burnt forests, the seeds were found to germinate profusely. But Tuggerse (1925b) found that in the burnt areas it was the old seeds that sprouted and not the seeds on the soil surface that were scorched by fire. Tuggerse (1925c) also described (1) the alternate soaking and baking method and (2) the method of burying the seeds for a specific period in the earth (these two are known as Burma methods). Dipping the seeds in a mixture of cow-dung and water for a week (known as the Cochin method) was also described but not appreciated by him. Tuggerse (1925d) got better germination in teak seeds by weathering them. In this simple method, the seeds are subjected to natural climatic conditions from the moment they are collected till they are sown after a year. The method was called 'open air treatment' and evaluated as the most inexpensive, practical and easiest one because any amount of seeds can be heaped on the ground where they can be frequently inspected for damage by insects and rats etc. Perfect aeration and easy turning are also possible.

Later on Chaturvedi (1942) described the success of his experiments on alternate wetting and drying the teak seeds in a seed bed. According to him, a basal layer mixture of grasses, twigs and branches etc. should be covered by a layer of sand, on which the seeds should be spread and covered by rich forest soil. The seed bed should be soaked in water every evening. The water is absorbed in due course by the soil and the seeds soon succumb and start germination. He further described the functioning and justification of all the above

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layers. Germinating seeds should be transplanted daily to the nursery.

Banerjee (1942) suggested a slightly different method for the above drying and wetting or cooling and heating treatment. He advocated drying seeds on a bamboo mat for a week. They should then be soaked in a water tank for 36–48 h. keeping them in small bags. These seeds should be dried for 12 h. and then wet again. The process of alternate wetting and drying should be continued for 2–3 weeks, and then the seeds sown in nursery beds. This method produced 80% germination.

Gopal et al. (1972) investigated removal of the felty mesocarp and soaking the seeds in a nutrient solution or in water to enhance the germination capacity. This was undertaken because it was apparent that a water-soluble germination inhibitor in the mesocarp was responsible for dormancy of seeds. This was assessed in seeds of 16 origins (Table 1), in which percentage germination of those seeds increased after being soaked in water for 24 h, during which the inhibitory nutrients leached out. Removal of the felty mesocarp resulted in germination. However, the best germination was shown by the seeds soaked in Sach's nutrient solution for 4 hrs. In general, seedlings started emerging 10–16 days after seed sowing and stopped after 31–56 days. But this period could be reduced by the treatment of seeds with Sach's nutrient solution (0.7g KNO_3, 0.25g $CaSO_4$, 0.25g $CaPO_4$, 0.25g $MgSO_4$, 0.08g NaCl and 0.005g $FeCl_3$ in one litre of water). Seedlings emerged from 7 to 14 days after sowing and emergence terminated between 26 and 31 days.

To enhance seed germination, Dnyansagar and Kothekar (1982) recommended a more modern technique of exposing the seeds to gamma rays at 15 KR. They found 30% germination of seeds due to the treatment as compared with only 8–10% in untreated seeds. The authors also observed the resultant seedlings for 2 years and found them fully normal in external as well as internal structure. This treatment is neither difficult nor expensive thus can be put to use even on a large scale. It was found that these exposures bring about early and rapid germination, due to shortening of seed dormancy or breaking it by one or more of the following four ways: (1) the ionising effect of radiation; (2) increasing permeability of the seed coat; (3) increasing formation of germination-controlling enzymes; (4) preventing disturbances in auxin synthesis responsible for germination. Most of the above methods are presently used in various parts of the country.

The impermeable woody endocarp is also a problem in germination of teak seeds. To study the importance of the stony endocarp, Dabral (1976b) used a method of extraction of teak seeds. In his experiment, he successfully tested a newly devised nut-cutter and found that the germination of extracted seeds was faster and easier than that of the fruit-enclosed seeds, which have to wait until the seedcoat and epicarp decay naturally.

Provenance trials

Extensive provenance trials have been undertaken in relation to teak. Kadambi (1945), while

Table 1. Percentage germination of teak seeds after certain pregermination treatments to the seeds of 16 provenances.

Provenances	Felty mesocarp removed	Seeds soaked in Sach's nutrient for 4 h	Seeds soaked in Water for 24 h	Control
Bairluty	16	25	9	1
Chinalpetti	38	45	30	23
Mohmedabad	5	4	1	21
Begur	100	104	91	86
Nilambur	35	44	39	31
Allapalli	0	14	4	1
Barhe	50	4	84	1
Deolpur	2	20	3	4
Khapa	5	19	2	2
Sindewahl	20	35	0	29
Cumbum	35	57	0	4
Manantvady	100	104.5	91	86.5
Topslip	21	37.5	27	27
Belgan	1.5	28.5	13	7
West Chanda	0	0	0	0
Nandiyal	0	0	0	0

investigating the pattern of tree growth, found that teak trees of Mysore and Shimoga exhibited very different morphological characters, even though the two places were neither distantly separated nor their climates very distinct from one another. Thus the significance of origin of teak seed was apparent. Using teak seeds from eight origins, Kadambi (1945) experimented on the inheritance of individual and racial characteristics and demonstrated that seeds of definite and known origins should be used for improving plantations. Later on Seth (1956) prepared a national progress report on seed and seed problems of teak, describing all the above factors. Using the seeds of 5 provenances from a wide selection of sites (Table 2), Gopal et al. (1972) found clearcut provenance variations in the number of useful and useless fruits. It was also found that seeds of moist localities germinated quicker than those from drier localities. However, Gupta and Pattnath (1975) found that teak seeds from dry areas germinate more easily than those from moist areas. Gupta and Kumar (1976) also studied the potential germinability of teak fruits from 23 sources using a cutting test, whereas Gopal and Pattnath (1980) indicated ways to improve seed production of teak in India.

During their provenance trials, Gopal et al. (1972) found that, on average, a bulk of seeds was useless (Table 2). Some of the fruits were completely seedless but others had unripe, ill-developed, immature or dormant seeds which could not germinate even after certain pregermination treatments. However, the number of seedlings was always slightly more than one per fruit.

During the provenance trials, effect of soil and sand on the germination behaviour of seeds of different origins was also studied. A consistently low germination percentage in teak prompted scientists to investigate the development process of fruits, starting from the formation of ovule.

Murthy (1973a) observed that there is a definite after-ripening period or after-ripening process in teak seeds. He noted that germination of early falling nuts was poor and such nuts have to undergo a second phase of ripening during storage, after which germination percentage approached that of the later-collected seeds which face daily drying in the sunlight. Effect of sun-drying on the germination capacity of seeds is apparent from the following data:

Tree location	*Germination (%)*
Full sunlight	26.8
Shade	4.5
Darkness	0.2

After-ripening was found to be a cause of delayed germination in teak. It was also observed by Murthy (1973b) that even the depth of insertion of seed in the soil has considerable influence on germination capacity in teak seeds, as indicated in Table 3.

Table 3. Influence of seed depth placement in soil on germination percentage.

Seed placement	Germination (%)
Fully above the ground	27.1
Half pushed into the soil	42.6
Fully pushed into the soil	46.3
One cm below the soil surface	22.8
2.5 cm below the soil surface	18.7
5.0 cm below the soil surface	7.0
7.5 cm below the soil surface	2.0
10.0 cm below the soil surface	0.5

Similarly, average germination percentage of big and small seeds also differed (48.1 and 36.5 respectively). However, it was noticed during a study that the age of the mother tree, after a certain limit, showed no effect on the germination potential of seeds in teak. Seeds from 31, 50 and

Table 2. Average percentage of seedlings and various kinds of fruits in 5 provenances of teak.

Provenance (State)	Seedlings obtained out of 100 fruits	Seedless Fruits	Fruit with dormant seeds	Total useless fruits	Total useful fruits
Manantavady (Kerala)	70.00	21.0	33.0	54.0	46.0
Topslip (Tamil Nadu)	28.0	19.0	60.0	79.0	21.0
Belgan (Orissa)	18.0	12.0	72.0	84.0	16.0
West Chanda (Maharashtra)	Nil	15.0	85.0	100	Nil
Nandiyal (Andhra Pradesh)	3.0	31.5	66.0	97.5	2.5

101 year-old trees germinated with same vigour.

Another factor affecting the germination potential is the physiological condition in the form of nutrient imbalance in the seed, brought about by the deficiency of stored nutrients of the soil. Gupta and Pattnath (1975) stated that such soil depletion is caused by teak itself whose power of absorbing the nutrients is very high. However, it returns only 8–10% of the total K and P to the soil. The idea was strengthened because from amongst the natural factors, fertility index of the site showed more pronounced effect on the seed behaviour than the others.

An important aspect investigated during the last few years is the performance of teak seeds in different potting media. Nursery technology for growing large numbers of seedlings from the seeds of a species and then transplanting them into the field, has become well-established for massive plantation programs worldwide. In this respect, it is essential to use the most suitable potting media for germinating the seeds as well as growing the seedlings in the initial phase. The medium may differ from species to species.

Pure, black natural soil was found most suitable for the growth of teak seedlings up to the age of 6 months. The development of roots in this medium supported better growth and establishment of the seedlings. This information can be used in the nurseries to obtain better seedlings of teak.

Emptiness of Fruits

Joshi and Kelkar (1971) were among the pioneers to investigate the process of fruit development in teak, and to investigate the phenomenon of seed dormancy in it. They demonstrated that from the four chambers of a fruit, not all were filled. Even if they were filled, not all the seeds were fully mature. They found that only one of the four seeds develop fully; the rest all remain underdeveloped. This was observed with very insignificant differences in fruits of different sizes (Table 4).

Table 4. Percentage of variously developed seeds in teak fruits of different sizes.

Fruit Size	Seed development			
	Full	Moderate	Ill	Rudimentary
Large	28.0	16.0	40.0	16.0
Medium	26.7	14.2	44.0	15.1
Small	24.5	12.0	50.0	13.5

According to Dabral and Amin (1975), partial or full emptiness of fruits depends upon the availability of food and nutrients to the developing fruits, but also largely upon the intensity of attack of teak skeletoniser of the inflorescence. However, Dabral (1976a) stated that there is no significant effect of the skeletoniser on the emptiness of fruits. After making a very comprehensive survey in a wide range of populations, he suggested that the emptiness occurs when the endocarp hardens due to heavy lignification in those loculi which have watery ovules. He found that in early stages, there is an ovule in each loculus. Reserve food material accumulated during the past growing season is utilised in seed formation and the emptiness of the chamber does not have any relation to the size of the chamber.

Prasad and Jalil (1986) determined the percentages of fruits having 4, 3, 2, 1 and 0 seeds for nine provenances of teak. Such information gives an approximate assessment of the expected number of seeds actually present in a fruitlot and such a number of seeds will be very near to the real plant percentage resulting from the fruitlot.

In their survey, Prasad and Jalil (1986) found that the percentage of 4-seeded fruits was almost negligible (1–2% in all the provenances collected from natural forests as well as teak seed orchards). The percentage of 3-seeded fruits was also very low, (only 2–6%). Large numbers (11–35%) were also found to be completely seedless. However, the percentage of fruits with only one seed per fruit was the highest (42–64%). Similarly, 2-seeded fruits were found to range from 12 to 25%. The authors suggested that finding out the average number of seeds per 100 fruits by cutting tests etc. and then finding out the viability of seeds, may give us an idea of the exact number of expected plants from a particular seedlot. Thus, we may not only save a lot of unnecessary expenditure but also can save a large amount of precious seed. Weathering of whole fruits and then sowing does not give any idea of how many seedlings may emerge from a seedlot. Using the following formula given by Dabral (1976a) the authors determined that on an average, emptiness per fruit varied in different provenances from 0.69 to 0.76. This indicates widespread emptiness — from 83 to 120 seeds per 100 fruits in different provenances.

$$\text{Average emptiness} = \frac{\text{Total number of empty loculi}}{\text{Total number of fruits} \times 4}$$

Seed Requirements for Plantation Programs

Selection and use of high quality seeds of superior genetic make-up was not a practice in the old days for any species. With the understanding

of the importance of such seeds in increasing the overall productivity of the plantations, more emphasis is now being given on this aspect. But, there is still no regular use of improved seeds of teak in India as there are no proper and adequate sources for getting sufficient quantities of high quality seeds.

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Recommendations and Reports

Recommendations for Research into Seed Problems of Multipurpose Trees and Shrubs

C. Schaefer, S.J. Midgley, P.B. Milimo and B.V. Gunn

THE meeting divided into four subgroups to discuss the major issues arising from the presentations. These were designated 'Seed Physiology', 'Seed Origin and Control', 'Exchange of Information' and 'Training'. The following recommendations were brought forward by each subgroup and approved by the final plenary session of the conference:

Seed Physiology

1. Procedure for Evaluating New Species

(a) A standard procedure be developed to test for viability and physical dormancy.
(b) Research on physical dormancy should be intensified in cooperation with plant physiologists and biochemists.
(c) Special attention be given to the influence of the pulp on species with fleshy seed coats.
(d) Species with a moisture content $\geq 25\%$ at seed fall should be tested for their recalcitrance.
(e) A seed vigour test should be developed to predict future seedling performance.
(f) A seed maturity index should be developed so that seeds can be harvested at the most appropriate time.

2. Storage of Tree Seed

Research on practical and economic ways of storage of tree seed should be given priority. In recognition of the importance of water relations to viability of all seeds, we recommend the following aspects for special attention:

(a) More work needs to be done on the correct methods for determining MC in seeds and, wherever possible, methods standardised for individual species.
(b) Assessing the suitability of ultra low moisture content storage (3–5%) of orthodox seeds should be a priority for research.
(c) For each important species, viability constants need to be determined. Care needs to be taken to avoid non-homogeneous and dormant seedlots. Also the temperature selected for estimation of constants needs to be precisely controlled.
(d) Many tropical tree seeds are recalcitrant and cannot now be stored. In recognition of the importance of these species, methods for storage should be given high priority.

Seed Origin and Control

1. IUFRO-associated tree seed centres to provide a defined minimum level of detail with regard to seed origin. This will include locality of origin, latitude, longitude, altitude, number of parent trees, date of collection and, where possible, optimum germination techniques.
2. The National Tree Seed Centre of Papua New Guinea to coordinate a postal survey to examine the possibility of standardising the format for the collection of origin data. They will take into acount forms currently recommended by OECD, FAO, DANIDA and other tree seed centres.
3. There is a basic need for taxonomic accuracy in seed collection and supply and any additional costs of taxonomic verification should be included in the cost of seed.
4. Due to the phytosanitary problems of seed exchange and the possibility of inadvertent exchange of pests and diseases, research on seed-borne pests and diseases should be viewed as an integral part of seed centre operations.

Exchange of Information

1. Proposals for funding to continue publication of the 'African Seed News' should be drafted by the current editor (with assistance from other regional subscribers) and sent to potential sponsors through the Seed Problems working group executive.
2. The 'African Seed News' should be distributed via a network of key regional contacts who will assist with contributions and, where possible, printing and/copying to reduce overall costs. It should also be translated into French to broaden the readership (this will be done by the delegates from Burkina Faso).
3. The Australian Tree Seed Centre should, through a periodic supplement to the ACIAR Forestry Newsletter, inform their clients of new developments and circulate feedback from users of Australian tree seed originating from the Centre.
4. The IUFRO working group on seed problems should give increased encouragement to exchange of information between seed workers.

Training

1. Increased input is required to identify training requirements and to coordinate these with training institutions and donors to develop efficient training programs.
2. There is a need for 'hands-on' training courses to be held in countries requiring staff training and consideration should be given to language problems.
3. There is a need to have technical publications made available in a wider range of languages.
4. There needs to be a general increase in training of forestry staff working with seed at a technical level. This should be followed by training at a graduate and postgraduate level.
5. Greater support should be given to staff exchange between organisations working on seed problems.

General Recommendations

1. The IUFRO Seed Problems project group should inform the Secretary-General of CILSS that it supports a regional program for strengthening seed centres in Sahelian-African countries and would be willing to assist in the organisation of training courses on seed problems.

2. Draft proposals to obtain funding from IDRC for regional workshops in Africa should be prepared by the African seed workers and passed through the working group executive for submission.
3. The importance of cooperation within and between organisations working with seed should be stressed.

A System for Classification of Seed Literature in Forestry

Final report of Project 4, IUFRO, Project Group P2.04-00

M. Simak*

Background

Seed workers are not only dealing with seed *in sensu stricto* but also with all stages and events occurring in the ontogenetic chain of seed formation: beginning with the induction of generative buds through flowering, sporogeny, pollination, fertilisation, embryogeny, fruit setting to seed itself and its germination process. Moreover, the trees on which this chain is operating as well as the offspring arising from the generative reproduction are included in the sphere of interest of the seed workers. They are studying the reactions of each step in the ontogenetic chain to various external factors, whether they are naturally or experimentally induced.

In order to approach all these different problems, the seed workers are compelled to use many scientific disciplines such as Botany, Chemistry, Ecology, Entomology, Genetics, Physics, Physiology, Silviculture, Technology, etc. This is, of course, an enormous working field and the literature related to forest tree seed is not only very comprehensive but also heterogeneous. It needs to be classified in such a way that the necessary information about each particular problem can be easily obtained.

Unfortunately, there is no bibliographical classification system which systematically covers the whole working field of forest seed science and technology. In the existing systems, 'forest seed' is either reperesented as small appendices to other forest disciplines (cf. The Oxford System of Decimal Classification for Forestry) or is included within the System for agricultural seed, which gives too little consideration to specific forestry problems (cf. ISTA-Bibliographical System). This is naturally a very unsatisfactory situation.

At the first symposium of the IUFRO-WP S2.01.06 'Seed problems' (now P2.04-00) held in Bergen, Norway, 1973, all these problems were discussed and in conclusion a desire was expressed to start a WP-project with the following goals:

1) To develop a standard bibliography system, which should entirely deal with the problems in forest tree and shrub seeds from all aspects particularly considering related forestry disciplines.

2) On the basis of this standard system gather systematically the bibliographic data on forest tree and shrub seeds in different countries and eventually establish a data base on these references.

In this connection about 15 delegates participating in the symposium agreed to compile the literature on forest seeds in their respective countries provided that such a system for literature classification was available. I was appointed to coordinate the project.

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Project History

At the Bergen symposium it was proposed that the same numerical key word-system as that accepted for the 'World Directory Project' could be used as a base for the Bibliography Project. However, it was soon found that this system was too limited for this purpose. Therefore, a new system was elaborated and presented as the 'SAF-system' at the WP S2.01.06 symposium in Japan 1976 (first draft). At this symposium and later by direct contact with seed workers (second draft) many valuable suggestions were gathered. After that the project was 'dormant' for several years for different reasons. Nevertheless, during this interval many problems in the project were solved and as a result the original, rather complicated SAF-system was simplified and replaced by the SA-system. On the basis of this SA-system a data bank on forest tree seed literature has been established at the Seed Laboratory, Dept of Silviculture, Swedish University of Agricultural Sciences, Umeå.

The bibliography includes 1615 references of scientific papers, popular science articles and research notes etc, which have been compiled systematically starting from the first year of publication from 35 of the best-known journals as well as other periodicals published in Sweden up to and including 1974 (Simak and Bergsten, Data base on forest seed literature published in Sweden up to 1975. Swedish University of Agricultural Sciences, Department of Silviculture, Report No. 23.) For the data-handling of the references, the PC-system 'REFED & REFPRINT' version 26 MA-DOS from Biosoft was used. All references are stored on hard disk and are easily accessible.

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The SA-system was presented and discussed under business session. It was concluded:

1) The establishment of a computerised data base on forest tree seed literature on an international level is desirable.
2) A complete SA-system will be distributed to the participants. Suggestions, if any, to the system should be sent to the author before the end of 1989.
3) The system should be published as soon as possible.

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