AN EXAMINATION OF THE VARIATION IN SEED GERMINATION RESPONSE TO VARIOUS LOW TEMPERATURE SEED STORAGE REGIMES

C.A. Gardiner

Australian Tree Seed Centre
CSIRO Division of Forestry
PO Box 4008, Queen Victoria Terrace
Canberra A.C.T. 2600, Australia

ABSTRACT

Samples of seed from the two closely related species with orthodox behaviour, *Eucalyptus urophylla* and *E. pellita*, were subjected to a variety of seed conditioning and low temperature storage regimes including several techniques using cryogenic methods (storage in liquid nitrogen at -196°C) as well as storage in mechanical freezers at -25°C and -85°C. Following storage the germination response was then examined using standard seed viability testing procedures for eucalypts. The two species showed different germination responses to the treatments. *E. urophylla* showed an increase in total germination (healthy and abnormal germinants) over the control in all but one treatment while total germination of *E. pellita* decreased from the control in all but one of the treatments. This result suggests that the germination response of a species following low temperature storage cannot be predicted with any certainty from the behaviour of closely related species. It appears that cryogenic storage may increase the percentage of abnormal seedlings in both species, a response not evident in the -25°C and -85°C temperature storage regimes.

1. INTRODUCTION

Long term storage of tree seed is becoming an important issue in world forestry today. Most species with orthodox seeds (i.e. those with relatively low moisture contents at maturity) are able to be stored for long periods at room temperature with minimal losses in seed viability. However numerous species with orthodox seed have a reputation for losing viability over time and can in some cases lose viability relatively quickly when stored under uncontrolled conditions (Boland et al., 1980). The rate of decline in seed viability varies considerably and is influenced by a range of factors including type of seed (orthodox or recalcitrant), moisture content and storage methods. In the case of eucalypts, storage life is shortest in *Eucalyptus deglupta*, 4 years at 23°C, (Doran et al., 1987) to over 30 years in the case of *E. camaldulensis*. Whilst low temperature storage (e.g. 4°C or -18°C), can greatly extend the storage life of orthodox seed, there is a need to extend this period further.

Current research indicates that low temperature storage (approx. -200°C), otherwise known as cryogenic storage, is one method which shows considerable potential for indefinite storage of seed. For the purposes of this paper cryogenic storage refers to the storage of seed in liquid nitrogen at -196°C. Storage in this way can be an important conservation tool for rare and endangered species as well as enabling the long term maintenance of valuable germplasm from natural populations of commercially important species. Long term storage of important germplasm from tree improvement programs for reference and comparative testing is another potential application.

While there have been many studies on the effects of cryogenic storage on seed of agricultural crops (Chin and Roberts, 1980); (Roos and Stanwood, 1981), there has been little corresponding work on cryogenic storage of forest tree seed. Agricultural seed tends to be genetically uniform and shows little variation within a seedlot. In contrast, seed collected from wild tree populations can show great genetic variation as most tree species are outcrossing and highly heterozygous (Sedgley and Griffin, 1989). When tree seed is to be stored for long term genetic conservation or preservation of improved seed it is desirable that all genotypes represented in the original seed collection are maintained. Previous studies have indicated that sub-zero temperatures can markedly increase the storage life of orthodox crop seeds, however little is known on the effects of cryogenic storage on forest tree seeds.

One of the most commercially important genera in world forestry today is *Eucalyptus* and species from this genus represent the world's most widely planted hardwood trees (Eldridge et al., 1993). Seed from this genus is generally easy to store and most species can maintain viability for many years when stored under low humidity at around 23°C (Boland et al., 1980). Some species, however, lose viability after only a few years at 23°C. Cryogenic storage may extend the storage life of such species, and will also be useful for long term storage of rare and endangered species. However it is important to examine the effects of cryogenic storage before the process can be successfully implemented. This pilot study examined the effects of various low temperature storage regimes on two closely related and commercially important species, *Eucalyptus urophylla* and *E. pellita*.

E. urophylla is a tall forest tree occurring naturally in eastern Indonesia on a number of islands in the Timor Sea including Timor, Wetar, Alor and Flores. It is one of only two species of *Eucalyptus* not occurring in Australia and is of considerable importance in tropical forestry. It forms the basis of important forestry plantations in numerous tropical countries particularly in humid and sub-humid tropical climates with several months of drought (Eldridge et al., 1993). It is also widely used as a parent tree for producing interspecific hybrids such as *E. urophylla* x *grandis*, which is commonly planted in Brazil.

E. pellita occurs in warm humid areas of north Queensland, Australia, the Western Province of Papua New Guinea and adjacent areas of Irian Jaya. It is becoming increasingly important in tropical forestry being planted in areas of high summer rainfall and a long dry period. It has also been used as a parent in important interspecific hybrids with *E. grandis* and *E. urophylla* (Eldridge et al., 1993).

Experience at the Australian Tree Seed Centre has shown that seed of *E. urophylla* loses viability quickly at room temperature and must be kept at approximately 4°C for medium term storage (10 - 20 years). *E. pellita* will store adequately for this length of time at room temperature (approx. 23°C). This suggests that the seed physiology of these two closely related species may be quite different and hence they will have different requirements for longer term storage.

A total of 12 provenances of *E. urophylla* and 6 provenances of *E. pellita* were examined (see Table 1). This enabled a comparison to be made between two closely related species as well as an examination of provenance variation within species. The results give an indication as to whether it is possible to predict the response of one species from the behaviour of another closely related species and whether the response is likely to be consistent throughout a range of provenances within a species. A variety of seed ages were included to enable an investigation into the effects of seed age in response to the various storage regimes.

Table 1. Provenance Origins of Seedlots Tested

CSIRO Seedlot	Location	Latitude (°'S)	Longitude (°'E)	Altitude (m)	Year Collected
Eucalyptus urophylla					
8238	Timor, IND	8 30	126 00	910	1963
8239	Timor, IND	8 30	126 00	1300	1963
10138	Mt Tatamailau, IND	8 55	125 30	2790	1971
10140	Hato Bulico, IND	8 53	125 32	2100	1971
13828	West Timor, IND	9 34	124 17	1200	1982
17831	Ilwaki, Wetar Is, IND	7 52	126 27	515	1990
17835	Carbubu, Wetar Is, IND	7 56	125 53	175	1990
17839	Apui, Alor Is, IND	8 17	124 40	1115	1990
17841	Piritumas, Alor Is, IND	8 19	124 31	355	1990
17843	Baubillatung, Pantar Is, IND	8 20	124 02	285	1990
18094	Mt Egon, Flores Is, IND	8 40	122 27	475	1990
18096	Lomblen, IND	8 23	123 32	515	1990
Eucalyptus pellita					
16615	Keru - Kumbalusi, PNG	8 35	141 45	35	1988
17854	Bupul - Muting, IND	7 21	140 36	40	1990
17861	NW Kuranda, QLD	16 41	145 32	440	1987
17874	Lankelly Creek, QLD	13 53	143 16	500	1990
17875	Mt Tozer, QLD	12 44	143 12	100	1990
18314	Tully, QLD	17 50	146 03	50	1991

IND - Indonesia, PNG - Papua New Guinea, QLD - Queensland, Australia

2. MATERIALS AND METHODS

Eucalypt 'seed' sold commercially usually consists of a mixture of fertile seed and unfertilized ovules known as chaff and these structures are similar in appearance (Boland et al., 1980). In most species of *Eucalyptus* it is possible to separate fertile seed from chaff although in many cases it is often difficult to identify all the fertile seeds in a given sample. In practice however it is a time consuming and costly process and is generally not undertaken except where automated sowing is a requirement. Therefore viability figures for *Eucalyptus* seed are expressed as the number of viable seeds in a 10 gram sample rather than as a percentage. The number of viable seeds per unit weight of seed can vary greatly within a species depending on the success of fertilization (Eldridge et al., 1993). Thus this method of expressing viability gives the seed user a clearer picture of the quantity of seed needed for his or her purpose.

Small representative samples of seed (including chaff) from each seedlot were subjected to the various low temperature storage regimes shown in Table 2. The sample weight used for the *E. urophylla* seedlots was 0.1 grams per dish and 0.2 grams per dish for *E. pellita*. The seed was then tested for viability using standard Australian Tree Seed Centre seed testing procedures. Samples of seed were sown on moist filter paper placed on a substrate of coarse vermiculite in 9 cm petri dishes. Four replicates of each seedlot-by-treatment combination were examined to provide an estimate of experimental error. The petri dishes were placed in controlled conditions at 25°C and received approximately 16 hours of artificial light per day.

Germinants were counted after they reached a sufficient size to enable a complete assessment of all their structures. Each time the seedlot was examined, fully developed germinants were removed and the number of germinants was recorded along with the number of abnormal seedlings. The seedlots were examined initially after approximately 7 days and then repeatedly every 3-4 days. Tests were terminated after 3-4 weeks.

During germination tests of *Eucalyptus* seed it is not uncommon to record a small percentage of abnormal germinants (Boland et al., 1980). These seedlings are generally excluded from viability figures as most are likely to die before reaching the advanced seedling stage. A *Eucalyptus* germinant consists of three basic structures, the radicle, the hypocotyl and the cotyledons. Abnormalities can occur in one or more of these structures. Table 3 shows the average number of germinants (including abnormals) for each treatment as well as the number of germinants which were considered to be abnormal. The standard viability of the seed (expressed as the number of normal germinants per 10 grams of seed) is also shown.

Table 2. **Treatments Used in the Low Temperature Storage Trial**

1. Control

Samples were sown directly with no pre-treatment.

2. Rapid Cool / Rapid Warm

Samples were placed in 1 ml NUNC cryovials which were then stored in cryocanes and dipped directly into liquid nitrogen. After three days the canes were removed and dipped directly into a hot water bath at approximately 40°C.

3. Slow Cool / Slow Warm

Samples were placed in 1 ml NUNC cryovials which were then stored in cryocanes and placed into a programmed freezer. The samples were cooled at 0.5°C per minute from 15°C to 0°C and then at 0.1°C per minute from 0°C to -40°C. Samples were then transferred directly to a freezer at -85°C for two hours prior to dipping in liquid nitrogen. After three days in storage the samples were removed and re-warmed in the reverse order of the cooling regime.

4. Slow Cool / Rapid Warm using Cryoprotectants

Samples were placed in 1 ml NUNC cryovials to which 1 ml of cryoprotectant was added. The cryoprotectant used was a mixture containing a 15% solution of Dimethylsulfooxide (DMSO) and a 10% solution of dextrose. The samples were allowed to soak in this solution for approximately 24 hours before being placed into a programmed freezer. The samples were then cooled at 0.5°C per minute from 15°C to -40°C and then transferred directly to a freezer at -85°C for two hours prior to dipping into liquid nitrogen. After three days in storage the samples were removed and re-warmed by dipping the samples into a hot water bath at approximately 40°C.

5. Storage at -196°C for an extended period

Samples underwent the same treatment as in treatment 2 however the samples were stored in the liquid nitrogen for 58 days before being re-warmed in a hot water bath at 40°C.

6. Storage at -25°C for an extended period

Samples were placed in 1 ml NUNC cryovials which were then placed directly into a freezer at -25°C. Samples were stored for 58 days before removal and were allowed to re-warm to room temperature on the laboratory bench.

7. Storage at -85°C for an extended period

Samples were placed in 1 ml NUNC cryovials which were then placed directly into a freezer at -85°C. Samples were stored for 59 days before removal and were allowed to re-warm to room temperature on the laboratory bench.

TABLE 3
Germination of Seedlots following Experimental Procedures

	Treatment	CSIRO SEEDLOT *Eucalyptus urophylla* 8238	8239	10138	10140	13828	17831	17835	17839	17841	17843	18094	18096	Av.	*Eucalyptus pellita* 16615	17854	17861	17874	17875	18314	Av.
Mean number of germinants per dish	1	32.0	39.3	32.8	12.5	26.0	65.0	57.8	24.0	104.3	83.5	106.8	50.0	52.8	71.5	89.5	111.0	67.5	82.0	129.0	91.8
	2	34.3	54.8	32.5	19.0	40.0	107.0	84.8	36.8	93.8	80.8	116.5	61.0	63.4	32.8	21.3	75.8	41.5	41.8	59.0	45.4
	3	32.3	47.3	31.3	12.3	24.0	81.0	56.8	33.5	72.8	85.0	110.5	48.5	52.9	73.5	81.8	102.0	55.8	60.8	96.3	78.4
	4	0.0	0.0	0.0	0.5	6.5	13.3	0.0	2.3	15.3	7.0	19.3	10.0	6.2	21.5	49.8	72.5	27.0	26.5	38.5	39.3
	5	33.8	55.5	32.3	15.0	32.8	77.3	75.3	32.8	97.3	78.8	104.5	52.3	57.3	30.3	19.8	69.8	40.0	41.5	61.3	43.8
	6	48.5	64.8	34.0	26.3	38.0	115.8	82.8	38.8	87.5	89.3	124.8	77.0	69.0	56.8	83.5	101.8	63.5	66.3	98.5	78.4
	7	32.8	46.3	24.3	23.3	36.8	107.0	85.5	43.0	91.0	81.0	113.8	63.8	62.4	90.3	117.3	112.8	50.5	69.5	139.3	96.6
Mean number of abnormal germinants per dish	1	2.0	3.8	5.5	1.0	1.0	1.3	1.5	0.8	1.5	1.8	2.5	0.5	1.9	6.3	3.8	5.5	4.5	3.5	3.5	4.5
	2	14.0	21.0	14.8	8.3	13.8	26.3	20.0	12.3	25.8	21.8	30.8	10.0	18.2	8.0	5.3	9.3	7.8	8.3	7.8	7.7
	3	6.5	11.5	12.5	3.3	1.0	3.3	4.3	4.5	11.0	6.0	8.5	6.5	6.6	7.5	15.8	8.8	13.5	4.5	9.3	9.9
	4	0.0	0.0	0.0	0.5	5.5	9.5	0.0	2.3	10.8	6.3	15.5	8.8	4.9	11.3	23.0	34.5	14.0	13.3	18.3	19.0
	5	12.5	22.8	12.0	5.4	9.2	24.0	19.6	9.2	28.2	22.9	23.0	10.5	16.6	8.5	4.8	14.0	9.2	10.8	11.6	9.8
	6	3.3	16.0	8.5	4.3	3.5	4.5	4.3	3.8	4.5	2.5	4.0	3.3	5.2	3.3	2.3	4.3	3.5	3.5	2.3	3.2
	7	5.3	22.0	8.0	4.8	2.8	5.8	6.8	5.8	8.3	4.0	5.8	8.0	7.3	7.8	3.8	7.8	3.0	5.0	5.8	5.5
Viability (/10g) (excluding abnormals)	1	3000	3555	2730	1150	2500	6375	5630	2325	10280	8175	10430	4950	5092	3263	4288	5275	3150	3925	6275	4363
	2	2030	3380	1775	1070	2625	8075	6480	2455	6805	5905	8575	5100	4523	1240	803	3328	1688	1678	2563	1883
	3	2580	3580	1880	905	2300	7775	5255	2900	6180	7900	10200	4200	4638	3300	3303	4663	2115	2815	4353	3425
	4	0	0	0	0	100	380	0	0	455	75	380	125	126	513	1340	1900	650	663	1013	1013
	5	2129	3275	2035	960	2362	5334	5572	2362	6908	5595	8151	4184	4072	1091	752	2792	1540	1536	2483	1699
	6	4525	4880	2550	2205	3450	11130	7855	3505	8300	8680	12080	7375	6378	2678	4063	4878	3000	3140	4813	3762
	7	2755	2430	1630	1855	3405	10125	7875	3725	8275	7700	10805	5580	5513	4128	5678	5253	2375	3225	6678	4556

TREATMENTS 1. : Control
2. : Liquid Nitrogen for 1 week
3. : Liquid Nitrogen (Slow Cool / Slow Warm)
4. : Liquid Nitrogen (Slow Cool / Slow Warm using Cryoprotectants)
5. : Liquid Nitrogen for 8 weeks
6. : Storage at -25°C
7. : Storage at -85°C

3. RESULTS AND DISCUSSION

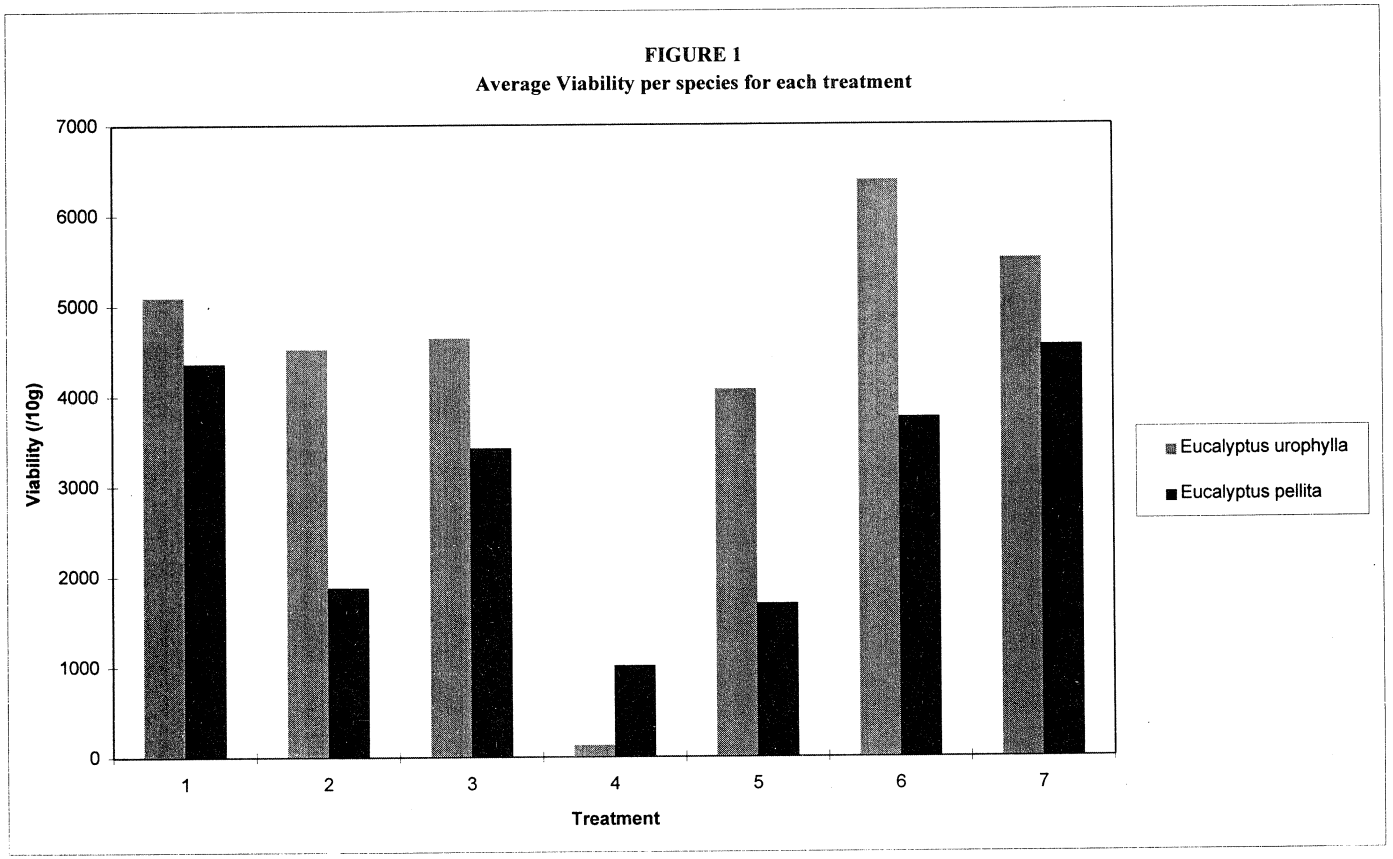
The results of the *Eucalyptus* storage trial are summarised in Table 3 and show that *E. urophylla* and *E. pellita* did not respond in a similar fashion to the various long term seed storage regimes. The average germination (including abnormal germinants) increased from the control in all but one treatment in *E. urophylla* while the average germination decreased from the control in all but one treatment in *E. pellita*. See also figures 1 and 2.

The increase in average germination in *E. urophylla* from the control seems to be primarily due to an increase in the number of abnormal germinants. However there is also evidence to suggest that in some seedlots of *E. urophylla* the freezing process may stimulate the germination of normal as well as abnormal germinants. Some seeds apparently remain dormant under standard germination conditions, and this dormancy may be broken following the freezing process. This apparent dormancy may be the result of a hardening of the seedcoat which is then weakened by the freezing process enabling the seed to imbibe. Alternatively it could be a form of embryo dormancy which is broken by the freezing process. This phenomenon was particularly evident following storage at -25°C and was encountered in most seedlots. Further work will investigate if these germinants are likely to have reduced vigour and thus be unlikely to survive to the seedling stage. Interestingly the two seedlots which showed a marked increase in viability following treatment 2 (liquid nitrogen for three days) were both from the island of Wetar, suggesting that there may be some provenance variation in this breaking of dormancy. Subsequent to this study, trees from the island of Wetar have been described as a separate species, *E. wetarensis* (Pryor et al., 1995). A similar increase in germination following low temperature storage of orthodox seeds not normally requiring treatments to break seed dormancy was encountered by Touchell and Dixon (1993).

The results from some seedlots suggest that some low temperature storage regimes may also induce abnormalities in otherwise normal seeds of *E. urophylla*. This seemed to be more evident in older seedlots and more pronounced in the rapid cooling regime (treatment 2) than the slow cooling regime (treatment 3). This suggests that older seedlots have a decreased ability to withstand cryogenic storage and that cooling rate is an important factor in freezing orthodox seeds. Similar abnormalities following rapid cooling regimes, recorded in orthodox crop seeds (Stanwood and Bass, 1981), were overcome by cooling at a slower rate.

For *E. pellita* there was a reduction in the total germinants from the control treatment in all treatments except storage at -85°C, and this reduction was most pronounced in the treatments using liquid nitrogen. There was an increase in the percentage of abnormal germinants in these treatments, however it would seem that the reduction in viability resulted mainly from the death of seed following storage in liquid nitrogen. The rapid cooling regime (treatment 2) lowered viability much more than did slow cooling in liquid nitrogen (treatment 3). While the germination in treatment 3 was not as high as in the control treatment, the result indicates that the rate of cooling is an important factor in the cryopreservation of *E. pellita*. Different cooling regimes may result in a better response to storage in liquid nitrogen for this species. The cooling rate may also be an important consideration when storing *E. pellita* in mechanical freezers.

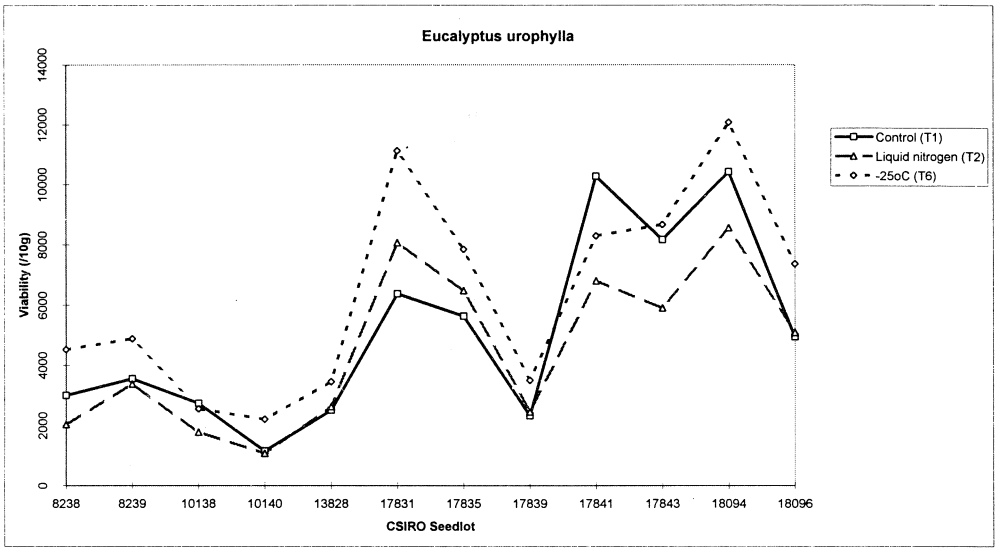
FIGURE 1
Average Viability per species for each treatment

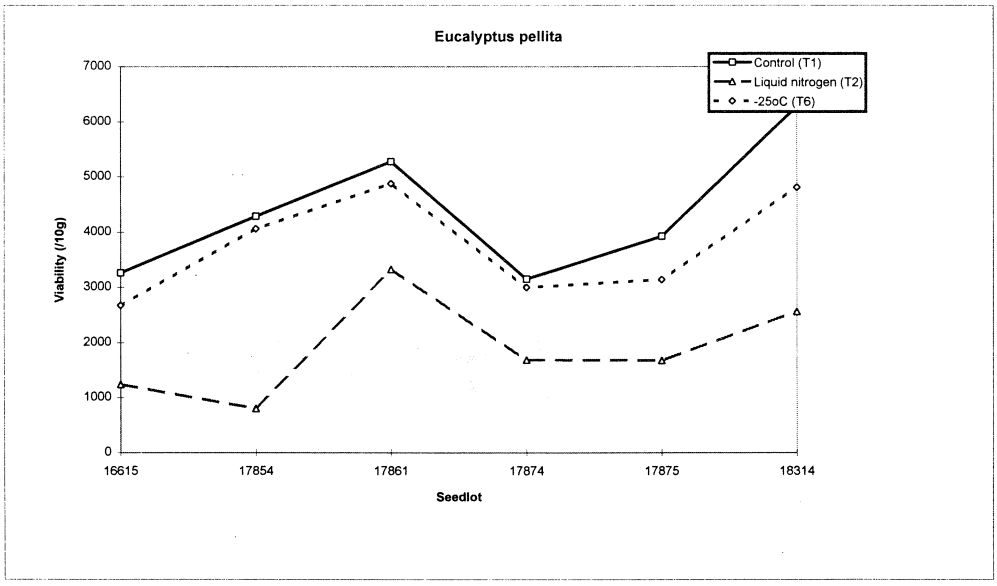


TREATMENTS

1. : Control
2. : Liquid Nitrogen for 3 days
3. : Liquid Nitrogen (Slow Cool / Slow Warm)
4. : Liquid Nitrogen (Slow Cool / Slow Warm using Cryoprotectants)
5. : Liquid Nitrogen for 8 weeks
6. : Storage at -25°C
7. : Storage at -85°C

FIGURE 2
Viability of Individual Seedlots - Selected Treatments





The failure of the treatment using the cryoprotectants Dimethylsulphoxide (DMSO) and dextrose was likely to have been a result of excessive soaking time allowing the seed to imbibe rather than toxic effects of the chemicals. A number of previous studies using DMSO have used soaking periods between 12 and 24 hours (Chin and Roberts, 1980) however most were performed on recalcitrant seeds. Other studies on orthodox seeds have recommended soaking times of as little as one hour (Grout and Crisp, 1985; Pence, 1991). The concentration of 15% DMSO is also common in other tests so the failure is unlikely to be a result of excessive concentration of DMSO. After soaking, the seed was washed thoroughly with distilled water. However, the seed was washed following placement on the germination test filter paper so it is possible there was some residual chemical in the filter paper. However it is considered more likely that the damage occurred during the freezing process and was a result of raising the moisture content of the seed. If the use of cryoprotectants is deemed necessary in the storage of *Eucalyptus* seed, different techniques will need to be investigated including greatly reduced soaking times. This study did however serve to further highlight the variation in response between *E. urophylla* and *E. pellita* as the effects were more severe in *E. urophylla* than *E. pellita*.

A number of the long term storage regimes seemed to induce fungal growth on seed in both species of *Eucalyptus* during germination tests. The fungal growth was more evident in treatments involving storage in liquid nitrogen and was extremely severe in the treatment using cryoprotectants, possibly as a result of the fungal spores present on the seed feeding from the dextrose solution used in the cryoprotectant solution. It would therefore be wise to consider treating the seed with fungicide prior to long term storage. Further work may be needed to study the interaction of fungicide treatments and long term storage regimes.

4. CONCLUSIONS

Results from this pilot experiment show that the response of a species following a long term storage regime cannot necessarily be predicted with any certainty from the behaviour of related species. Even closely related species may have different seed physiology and hence require different storage conditions. The results suggest that storage in mechanical freezers at -25°C or -85°C may be more appropriate than cryogenic storage for long term storage of some orthodox seeds although the longevity of seed in these treatments needs to be examined further.

The study also highlighted the importance of cooling rate when freezing orthodox seeds and this should be examined when designing a storage regime for a particular species. It also indicated that the response of a species to a particular storage regime may depend on the age of the seed and there may also be differing responses between provenances of the same species. Thus it is likely that individual storage techniques may need to be investigated for each individual seedlot to ensure maximum germination response to long term storage.

5. ACKNOWLEDGEMENTS

The author wishes to acknowledge the support of the Japanese Government and particularly the Forestry and Forest Products Research Institute for the opportunity to undertake this research project in Japan. I would also like to acknowledge the encouragement and support offered by the Australian Tree Seed Centre and the CSIRO Division of Forestry in enabling me to spend time in Japan and undertake this study. Ms Sarah Whitfeld assisted with analysis of the data and Dr Chris Harwood and Mr Brian Gunn provided useful comments on the text.

6. REFERENCES

Boland, D.J., Brooker, M.I.H. and Turnbull, J.W. 1980. Eucalyptus Seed. CSIRO Australia.

Chin, H.F. and Roberts, E. H. 1980. Recalcitrant Crop Seeds. Tropical Press SDN. BHD. Malaysia.

Doran, J.C., Turnbull, J.W. and Kariuki, E.M. 1987. Effects of Storage Conditions on Germination of Five Tropical Tree Species. In proceedings IUFRO Symposium on Forest Seed Problems, Harare, August 1987 (ed. S.K. Kamra and R.D. Ayling) pp. 84-94. Swedish University of Agricultural Sciences, Umea.

Eldridge, K.G., Davidson, J., Harwood, C.E. and van Wyke, G. 1993. Eucalypt Domestication and Breeding. Oxford Science Publications, U.K.

Grout, B.W.W. and Crisp, P. 1985. Germination as an unreliable indicator of the effectiveness of cryopreservation procedures for imbibed seeds. Annals of Botany 55, 289-292.

Pence, V.C. 1991. Cryopreservation of seeds of Ohio native plants and related species. Seed Sci. and Technol., 19, 235-251.

Pryor, L.D., Williams, E.R. and Gunn, B.V. 1995. A Morphometric Analysis of *Eucalyptus urophylla* and Related Taxa with Descriptions of Two New Species. Australian Systematic Botany, 8, 57-70.

Roos, E. and Stanwood, P.C. 1981. Effects of low temperature, cooling rate, and moisture content on seed germination of lettuce. Journ. of the American Soc. of Hort. Sci. 10G (1) 30-34.

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

Stanwood, P.C. and Bass, L.N. 1981. Seed germplasm preservation using liquid nitrogen. Seed Sci. and Technol., 9, J33-437.

Touchell, D.H. and Dixon, K.W. 1993. Cryopreservation of seed of Western Australian native species. Biodiversity and Conservation 2, 594-602.

Vertuccg, C.W. 1989. Effects of cooling rate on seeds exposed to liquid nitrogen temperatures. Plant Physiology 90, 1121-1128.

INTERNATIONAL STANDARDS AND THE TESTING OF TROPICAL TREE SEED

Peter G. Gosling

Forestry Commission Research Station,
Alice Holt Lodge, Wrecclesham
Farnham, Surrey, GU10 4LH, UK

ABSTRACT

Accurate, reproducible and standardised seed sampling and testing procedures are vital for measuring seed quality, increasing efficiency in crop production and serving as the basis for national and international laws to facilitate seed trading.

The primary aim of the International Seed Testing Association (ISTA) is to promote the use of seed sampling and testing procedures embracing the above principles. International rules for seed testing, plus numerous handbooks are published and regularly updated by ISTA to fulfil this role, but the main emphasis has been on the seeds of agricultural and horticultural crops, temperate trees and flowers. Nevertheless, the seed characteristics measured and the principles used to achieve accuracy and reproducibility form an ideal basis for the testing of other seeds such as tropical trees and shrubs. This paper will discuss the application of international standards to tropical tree seed sampling and testing.

Keywords: Sampling, moisture content-, purity-, seed weight-, germination, viability-, cut-, x-ray-, tetrazolium-, excised embryo- tests, dormancy, pretreatment.

1. INTRODUCTION

The history of seed testing has been comprehensively reviewed by Steiner (1994), MacKay (1972) and Justice (1972). Baldwin (1942) gave the subject a tree seed perspective. The milestones are as follows:-

The first laws regulating seed trade and requiring the quality of seed to be shown on a label were passed in Switzerland in 1861. But it was not until 1869 that Frederich Nobbe established the worlds first seed testing station in Tharandt, Saxony, Germany. Standardised seed testing rules were first published in the USA in 1897, and in 1908 seed analysts from the USA and Canada founded what was to become the Association of Official Seed Analysts (AOSA). The International Seed Testing Association (ISTA) was formed in 1921 and adopted its first set of seed testing rules in 1931.

However, seed testing rules are never static. Seed analysts, scientists, technologists, processors, merchants, growers and legislators are constantly working together to improve the accuracy and reproducibility of existing seed sampling and testing methods and extend the benefits of standardised methods to new species. Nowadays, emphasis is also being increasingly placed on performing the same or modified tests more cheaply and without compromising accuracy.

This paper will begin with a consideration of the importance of sampling as a prelude to seed testing. And then present a brief summary of several types of seed test under the headings of 'Objective(s) and general principle(s) of test' and 'Application of results and specific considerations for tropical tree species'.

2. SAMPLING

The size of a sample taken for seed testing is often very small in comparison to the seed lot from which it is drawn. It must therefore be as representative of the bulk as is humanly possible. No matter how accurate the subsequent technical work at the seed testing laboratory, the results can only show the quality of the submitted sample. The stock to be sampled must therefore be mixed as uniformly as practical and the sample taken to accurately reflect the composition of the bulk.

In practice, the various components of a seed bulk (pure seed, other seed and inert matter) are rarely uniform throughout and it is therefore wise to subsample from different parts of the bulk, combine the sub-samples and mix a so-called composite sample.

The size of sample submitted for testing can also depend on the size of individual seeds, the number and type of test(s) required and the value of the seed.

In the case of moisture content samples 2 x 5g samples are almost universal.

As a 'rule of thumb' samples for purity and germination/viability testing should contain about 5000 pure seeds. However, in the case of very small seeded species (eg *Eucalyptus* spp.) 5000 seeds may weigh so little that a sample of this size is impractical - in these cases although samples of 1-5g may contain tens of thousands of seeds, they are usually accepted as a reasonable compromise. With very large seeded species, a sample containing 5000 seeds may become impractically large. It is then common to adopt a sample size of 1kg, so long as it contains between 500-5000 seeds. Where seeds are even bigger, it is unwise to reduce the sample below 500 seeds, even if the sample weighs more than 1kg.

It is also important to prevent if possible, or at least minimise, changes in sample quality between sampling and testing. Obvious things to ensure are that moisture content samples are transported in moisture proof containers, and that purity and germination samples are neither crushed nor overheated.

3. TESTS OF PHYSICAL QUALITY

3.1 Moisture content test

Objective(s) and general principle(s) of the moisture content test

The objective of this test is to determine the moisture content (mc) percentage (by weight) of a seed sample, (and by inference the mc of the seedlot). This value is usually reported on a fresh weight basis (f. wt) but can also be reported on a dry weight basis (d. wt) - beware of the difference.

The general principle of moisture content determination is that most methods rely on the assumption that heating seeds will drive off moisture. Therefore by drying seeds to constant weight, any reduction in weight will reflect water loss. For seeds containing significant amounts of volatile substances such as resins, fats, oils etc. this is slightly erroneous because these substances are also driven off by heating. However, even for these species the error is usually relatively small, and if the maximum temperature is kept to 105°C and heating stopped at between 18-24 hours, then discrepancies are of little more than academic importance.

Application of results and specific considerations for tropical tree seeds

Seed moisture content is important for several reasons. Moisture content is critical in determining storage potential and likely longevity - though care must be taken to ensure that the differences between 'orthodox' and 'recalcitrant' seeds are taken into account. 'Orthodox' seeds (ie those that can be dried and stored) should possess a low moisture content, say 5 - 10 % (f.wt basis). Whereas the desirable moisture content of so-called 'recalcitrant' seeds (ie those where drying, and sometimes temperatures below 15°C, are likely to kill seeds) varies between species. Some recalcitrant species begin to die as mc decreases from 60 %, others are not susceptible until mc's decrease below 40%. In the case of orthodox seed a low moisture content is a good sign because you are not paying for water, the seed is less likely to have deteriorated before you receive it and it should be in a good condition for storage. In the case of recalcitrant seeds a low moisture content is a bad sign, you are probably buying dead seed.

The ISTA method for determining the mc of tree seeds is basically i) weigh seeds; ii) incubate seeds at 103°C for 17 +/-1 hours; iii) re-weigh seeds; iv) calculate % mc.

In the case of large tree seeds or those with a very hard seed coat or fruit case, it is recommended that the mc determination is applied to a minimum of 5 seeds cut into small pieces and weighed as quickly as possible (to avoid drying) see for example Bonner (1991).

3.2 Purity test

Objective(s) and general principles of the purity test

The objective of this test is to determine the percentages (by weight) of 'pure seed', 'other seed' and 'inert matter' in a sample, (and by inference the composition of the seed lot). Also to identify the other seeds and inert matter.

The general principle of purity testing is to meticulously separate the sample, (often by hand), into three fractions - pure seed, other seed and inert matter, and then weigh the separate fractions. Each component is reported as a percentage of the total weight. Only the pure seed fraction is used in subsequent tests.

Application of results and specific considerations for tropical tree seeds

Commercial transactions of seed are nearly always conducted by weight. Hence, it is essential to know the percentage (by weight) of pure seed when comparing prices. The weight and nature of any contaminants is also important for a variety of reasons. A high percentage of other seed is clearly undesirable, but paying more for seed with a low other seed % may be an unnecessary luxury. Firstly, 'other seed' can only ever produce a rogue plant if it is alive - and this is not usually part of the seed test! Secondly, a 0.1% (by weight) contamination with small seeds such as *Eucalyptus* could constitute a very large number of plants; but a similar weight of larger seeds may be insignificant. The scale of the two problems is very different. Similarly, the consequences of a high percentage of inert matter depend on the method of plant propagation to be used. Large quantities of inert matter may be immaterial if seed is to be broadcast sown. Providing that allowances are made in sowing density calculations and a uniform distribution of pure seed : impurities is maintained throughout sowing operations, then purity percentages are of little practical significance. However, this is in distinct contrast to the plant propagator involved in container production. The substitution of inert matter for pure seeds in this system will result in 'blanks', much wasted space and a thoroughly uneconomic product.

3.3 Seed weight test

Objective(s) and general principle(s) of the seed weight test

The objective of the test is to measure seed weight in a unit which enables meaningful comparisons between seed lots and species. The standard unit adopted is the 'thousand pure seed weight' (tpsw), usually reported in grams.

The general principle is that pure seed separated during the course of the purity analysis is counted into replicates of e.g. 100 seeds and each replicate weighed.

The 100-seed samples prepared in the seed weight test also provide the replicates of counted seed which can be used for any of the germination or viability tests described later.

Application of results and specific considerations for tropical tree seeds

Seed weight is a function of seed size and average density. Differences in tpsw can reflect differences in seed filling caused by latitude and/or altitude and hence give an indication of seed origin/provenance. In addition the density of seeds is often influenced by the proportion of full : empty : insect damaged seed, and by seed moisture content. However, seed weight is often considered to be of most use when combined with the purity percentage and expressed as the 'number of pure seeds per kg' when it enables meaningful comparisons to be made between different seed lots.

4. TESTS OF PHYSIOLOGICAL PERFORMANCE

4.1 Germinability versus viability

Most forms of animals and plants which are visible to the naked eye are relatively easy to recognise as living or dead. This is not true of seeds. Hence there is a need for specific tests to assess their physiological status and likely performance.

There are broadly two types of seed test which do this. The 'germination' test as the name implies measures the proportion of seeds that are capable of germinating. The alternative is one of a range of 'viability' tests which permit seeds to be classified as either alive (=viable) or dead. It is vital to appreciate that viability tests are not a direct measure of the number of seeds capable of germinating and that 'viable' and 'germinable' are therefore not synonymous. Further discussion of the distinctions between these two types of test will appear later.

The choice between either a germination or viability test should ideally depend upon which test gives the best information. However, although the germination test nearly always fulfils this criterion (it is after all a direct measure of the characteristic which the nursery manager wishes to recreate in the nursery!), there are a number of reasons why a germination test may be impractical. For example, viability tests are usually much quicker than germination tests, and if there is a tight deadline to be met, there may be no alternative but to use a viability test. Other considerations include the following: Does the seed exhibit dormancy and need pretreatment? Is it known how to pretreat the seed? How long does seed pretreatment take? Are the optimal germination conditions known? How long does a germination test take? Do any methods of viability test exist for the species under consideration? Are there adequate indications that the viability tests described are reproducible? Have the results of germination and viability tests ever been compared for the species? It is rarely a simple matter to decide between these factors - but it is a choice frequently faced by seed testers. As a 'rule of thumb', germination tests are usually favoured and viability tests are generally applied for the following three reasons:

1. when it is known that dormant or slow germinating seeds will take more than 6 weeks to reach germination capacity.

2. when recalcitrant seeds are known to have a very short storage life.

3. whenever an estimate of physiological performance is required more quickly than the fastest germination test can be carried out.

4.2 Viability tests

Introduction

This section is structured differently to the preceding ones because there are several types of 'viability' test. It begins by describing the overall objectives and general principles of all viability tests, then considers four specific viability tests suited to tropical tree seeds (cut-, x-ray-, tetrazolium- and excised embryo- tests). The application of the results from the various different types of viability test is discussed at the end of the germination test section.

Overall objective(s) and general principle(s) of viability tests

The objective of this type of test is to determine the maximum percentage of viable seeds in a sample, (and by inference the seedlot), - by whatever technique is selected.

The general principle is to use the pure seed counted into replicates during the seed weight test and apply a single viability test to determine the percentage of live (=viable) and dead seeds according to whatever criteria are used in that particular viability test. Sometimes more than one viability test is used for comparative purposes.

Several types of viability test exist for tree species. Some of the most important include 'cut-', 'x-ray-', 'tetrazolium-' and 'excised embryo-' tests. When selecting between them, it is always necessary to consider at least four important features. Firstly, each viability test uses a different seed characteristic to indicate whether seed tissues are dead or alive. Secondly, there is often a degree of subjectivity in drawing a distinction between living and dead tissues. Thirdly, there is further subjectivity in interpreting whether areas of dead tissue are in important meristematic regions or are large enough to indicate an overall living or dead seed. Finally, since viability tests are only an indirect indicator of whether a seed is dead or alive, even seeds that are deemed to be alive, may not be capable of germinating because of dormancy, unsatisfactory pretreatment, or unfavourable germination conditions. Hence a percentage viability derived from one type of test may not be the same as a percentage viability derived from a different test.

A viable seed is therefore one which is best considered as being **alive according to whatever criteria are used in a particular viability test**.

4.2.1 CUT TEST

Objective(s) and general principle(s) of the cut test

The objective of the cut test is to determine the percentage of viable seeds within a sample - by cutting.

The general principle behind the cut test is that only full and physically undamaged seeds can ever have the potential to germinate. Therefore when seeds are cut open, only those which appear clean, firm and healthy may be viable. Those seeds which are entirely empty, or where the embryo appears undeveloped, shrivelled, mouldy or insect damaged are not viable.

The cut test is probably the simplest, oldest and crudest method of assessing the potential performance of seeds. It is not a method prescribed by the ISTA but is nevertheless so widely employed that any discussion of viability testing would be incomplete without giving it a mention.

Since the cut test is not described within the ISTA rules, but it is incorporated as a preliminary phase of testing *Aesculus hippocastanum, Castanea sativa and Quercus spp.* (three temperate recalcitrant species), it is worth mentioning that it is probably an especially useful technique for tropical recalcitrant species for two reasons. Firstly, it gives a rapid estimate of viability on seeds that are notoriously short lived. Secondly, cutting the fruits often facilitates the removal of the outer seed/fruit case and the resultant partially dissected fruits can be transferred to a suitable germination medium where germination is significantly faster than it is for intact fruits - so cutting forms an extremely useful prelude to a full

germination test on such species. Finally, since recalcitrant species have often sprouted before sample receipt, it is common to report the cut test in the following categories:

Total % viable = clean, firm and healthy embryos (whether sprouted or not)

Sprouted % viable = sprouted fruits

Total % dead = undeveloped, shrivelled, mouldy and insect damaged.

Insect damaged % dead = insect damaged fruits

4.2.2 X-RAY TEST

Objective(s) and general principle(s) of the x-ray test

The objective of the x-ray test is to determine the percentage of viable seeds within a sample -by x-ray.

The general principle is to use x-rays to reveal the internal structure of seeds. In the simplest case seeds are merely x-rayed without undergoing any preliminary treatment prior to x-raying and the varying shades of light and dark on a photographic image enable filled, empty, insect- and physically- damaged seed to be differentiated (see Figure 1).

Alternatively it is possible to use various contrast agents before x-raying. These permeate living and dead seed tissues to different degrees and the pattern, and depth of shading is interpreted in a similar fashion to TZ staining, as a means of distinguishing living from dead tissues, and hence potentially viable versus non-viable seeds (see for example Simak, 1991).

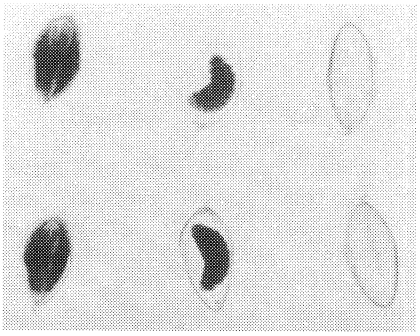


Figure 1. X-ray photo of conifer seed showing (l-r) full, insect damaged and empty seed

4.2.3 TETRAZOLIUM (TZ) TEST

Objective(s) and general principle(s) of tetrazolium test

The objective of the tetrazolium test is to determine the percentage of viable seeds within a sample - by tetrazolium.

The general principle of the TZ test relies on the premise that only living cells have the enzymes capable of converting a colourless, soluble compound (2,3,5 triphenyl tetrazolium chloride [TZ]) into an insoluble red product (2,3,5 triphenyl formazan). Seeds are therefore soaked in a colourless solution of TZ, the TZ enters both living and dead cells, but only the living cells catalyse the formation of the insoluble red precipitate. The pattern of stained versus unstained areas is then used to differentiate between living and dead tissues, and this is further interpreted as indicative of live versus dead seeds. Finally, there are some seed testers who claim to make an additional level of interpretation and relate certain staining patterns to normal germinants. In my own view, for the majority of tree seeds, the percentage of viable seeds in a TZ test is more likely to equate with the sum of 'normal germinants', plus 'abnormal germinants' plus 'fresh, ungerminated seed' at the end of a germination test. The TZ technique will take many years of refinement to differentiate between either 'normal' and 'abnormal' germinants; or 'non-dormant' and 'dormant' seeds of tropical trees.

4.2.4 EXCISED EMBRYO TEST

Objective(s) and general principle(s) of excised embryo test

The objective of the excised embryo test is to determine the percentage of viable seeds within a sample - by incubating excised embryos under conditions favourable for growth.

The general principle is to excise embryos from seed and incubate them under conditions favourable for growth. Embryos which either remain firm and fresh or show evidence of growth (eg expansion, elongation or greening) or growth and differentiation (eg radicle and lateral root formation; or epicotyl and first leaf formation) are considered viable. Embryos which show signs of decay are not viable.

4.3 Germination test

Objective(s) and general principle(s) of the germination test

The objective of this test is to determine the maximum germination potential of a sample (and by inference the seed lot). The result is usually reported as a maximum germination percentage (also known as a germination capacity; sometimes incorrectly referred to as a germination rate, a term which should be used only to indicate speed of germination).

The general principle is to use the pure seeds counted into replicates during the seed weight test and incubate them under a single, standard (preferably optimal) set of environmental conditions, to achieve the quickest, most uniform and complete germination possible for the majority of seed lots. At the end of the incubation period germinated seeds are classified as either 'normal-' or 'abnormal-' germinants; and ungerminated seeds as either 'fresh', 'dead' or 'empty'. Sometimes, in the case of so-called hard-seeded legume species, unswollen seeds at the end of the germination test may be reported as 'hard'.

When significant numbers of 'fresh' and/or 'hard' seeds remain at the end of a germination test, it is indicative that seed dormancy may be present. A full discussion of the dormancy phenomenon is outside the scope of this article but useful reviews include Bewley and Black (1994), Vegis (1964), Lang (1965). The most profound influence of seed dormancy on seed germination testing is the need to identify and apply a suitable pre-(germination) treatment (usually abbreviated to pretreatment), in order to stimulate complete and preferably prompt germination. Table 1 lists a few pretreatment procedures but unfortunately none are universally effective. Depending upon the likely presence or absence of dormancy there are three potential options available for germination testing.

1. A single germination test - **without pretreatment**. This should be applied to seedlots of species where there is virtual certainty that untreated seeds will germinate readily; i.e. dormancy is absent and pretreatment will not stimulate either germination capacity or rate.

2. A single germination test - **with pretreatment**. This should be applied to seedlots of species where there is virtual certainty that untreated seeds will not germinate at all, a successful pretreatment is available and has been proven essential to stimulate germination; i.e. dormancy is present in virtually all seedlots.

3. A 'double' or 'paired' germination test - **one with, plus one without pretreatment**. This consists of a 'control' or 'reference' germination test on untreated seeds, plus a germination test under exactly the same conditions after pretreatment, enabling comparison to be made between the two. 'Double' tests should be applied in all instances where there is doubt about whether the species or individual seedlot in question has dormancy.

A further variation on the standard germination test is the so-called 'weighed replicate' germination test. Germination tests are usually performed on a specified number of seeds, however, in some genera e.g. *Eucalyptus*, the seeds may be so small that it is impossible to distinguish pure seed from inert matter during the purity test. In other genera e.g. *Betula* it may just be too time-consuming to identify and count specified numbers of pure seed. And in yet other cases the effort of counting specified numbers of seeds may be unjustified because of very high percentages of empty seed. It is then permissible to weigh out seed samples that are likely to contain about 100 seeds and treat each as a germination test replicate. In the case of such weighed replicate tests, results cannot be reported as a germination capacity percentage but only as 'germinable seeds per kg'.

Table 1. Pretreatment techniques to overcome seed dormancy.

1.	Physical, chemical, or biological abrasion, degradation or puncturing of the seed coat, e.g. burning, acid, boiling/hot water, chipping.
2.	Treatment with plant growth regulators, e.g. gibberellic acid, ethylene, cytokinins.
3.	Chemical treatment, e.g. with KNO_3, KCN, NaOCl, H_2O_2.
4.	Prechilling of moist seed at 1-4°C.
5.	Alternate warm (ca 15-20°C) then cold (ca 1-4°C) treatment of moist seed.

Overall application of viability and germination test results and specific considerations for tropical tree seeds

Germination or viability test results are usually presented as a single percentage figure - germination capacity or viability percentage. Germination capacity is a direct measure of the characteristic which the nursery manager wishes to recreate in the nursery (that is germination), and is therefore likely to be most informative. A viability percentage is a less direct measure of germination and will need more careful consideration.

Care should also be taken to note whether a germination capacity was obtained with, or without a dormancy breakage pretreatment. Sometimes more than one value may be presented. This depends on whether 'double' germination tests have been applied; or even less commonly when more than one pretreatment has been used. The form of presentation should act as the first guide to whether seed dormancy is likely to be present, whether pretreatment is necessary and what sort of pretreatment may be desirable. Sometimes an analysis may have been made comparing not just the final germination capacity between pretreated and untreated seeds but also the respective rates of germination, this will give added information about dormancy levels (Gosling and Peace, 1990).

A viability percentage or the highest germination capacity (from multiple germination test results) is generally taken to reflect the **maximum potential performance** of the seedlot. Either value can be used to compare the germination or viability quality of different seedlots or assess the suitability of a seedlot for container, or precision sowing versus broadcast sowing. Clearly, where the germination capacity or viability of a seed lot is only measured at e.g. 50 %, sowing one seed per container is certain to be wasteful of space and containers!

Germination (and viability) percentages are also combined with purity and seed weight results to give an indication of the overall quality of a seedlot in terms of 'germinable (or viable) seeds per kg'. In common with germination capacity these are slightly different units which are appropriate for comparing the quality of one seed lot with another and calculating seed requirements. When seed prices are known, the relative value of different seedlots can also be compared. Finally, germinable (and viable) seed per kg provides an ideal starting point for calculating sowing densities for broadcast sowing programmes.

Correlation of laboratory viability and germination test results with nursery emergence

It is a common misconception that the objective of laboratory germination and viability tests is to predict nursery emergence! It has been shown above that this is not strictly so. A laboratory germination test measures the maximum germination percentage attainable by a seed lot under standard (often ideal) laboratory conditions. A laboratory viability test is an indirect measure of whether a seed is dead or alive. Hence germination capacity in the laboratory is often a significant overestimate of nursery seedling emergence and a viability percentage is prone to be an even bigger overestimate. The explanation is that tree seeds are notoriously sensitive to environmental conditions, nursery conditions are rarely as conducive to germination as the laboratory and the pretreatment of large nursery seedlots is rarely as effective as the pretreatment of smaller seedlots in a seed test.

5. CONCLUSIONS

The use of standardised procedures in sampling and testing seeds can ensure that results obtained on a sample from a given seed lot in one laboratory should not be significantly different from the results obtained on a similar sample, from the same seed lot, tested in any other laboratory. The seed characteristics described in the ISTA rules and the principles used for measuring them form an ideal basis for the testing of tropical trees and shrubs.

6. REFERENCES

Association of Official Seed Analysts. 1982. Rules for testing seeds. J. Seed Technol. 6, 1-126.

Baldwin, H.I. 1942. Forest Tree Seed. Chronica Botanica Co, Waltham, Mass., USA. pp 240

Bewley, J.D. and Black, M. 1994. Seeds: Physiology of development and germination. Second Edition, Plenum Press, New York.

Bonner, F.T. 1991. Measurement of moisture content. In: Tree & Shrub Seed Handbook. Eds. A.G. Gordon, P.G. Gosling and B.S.P. Wang. Chapter 12. International Seed Testing Association, Zurich, Switzerland.

Gosling, P.G. and Peace, A.J. 1990. The analysis and interpretation of ISTA 'double' germination tests. Seed Sci. and Technol. 18, 791-803.

International Seed Testing Association. 1993. International Rules for seed testing. Seed Sci. and Technol. 21, 1-288

Justice, O.L. 1972. Essentials of seed testing. In Seed Biology, vol 3, 301-370. Editor - T.T. Kozlowski. Academic Press, New York, USA.

Lang, A. 1965. Effects of some internal and external conditions on seed dormancy. In: Encyclopedia of Plant Physiology, 15, (2) 848-893.

MacKay, D.B. 1972. The measurement of viability. In: Viability of Seeds. Editor - E.H. Roberts. Syracuse Univ. Press, New York, USA.

Simak, M. 1991. Testing of forest tree & shrub seed by x-radiography. In: Tree & Shrub Seed Handbook. Eds. A.G. Gordon, P.G. Gosling and B.S.P. Wang. Chapter 14. International Seed Testing Association, Zurich, Switzerland.

Steiner, A.M. 1994. 125 years of seed testing. ISTA News Bulletin, 107, 24-25

Stokes, P. 1965. Temperature and seed dormancy. In: Encyclopedia of Plant Physiology, 15, (2) 746-803.

Vegis, A. 1964. Dormancy in higher plants. Ann. Rev. Plant Physiol. 15, 185-224.

PRETREATMENT AND GERMINATION OF ACACIA NILOTICA AND LEUCAENA LEUCOCEPHALA SEEDS

Peter G. Gosling, Yvonne Samuel and Steve K. Jones

Forestry Commission Research Station
Alice Holt Lodge, Wrecclesham,
Farnham, Surrey GU10 4LH, UK

Peter Gosling

ABSTRACT

The tip of a hot soldering iron (450°C) was found to be the easiest and most effective 'small scale' pretreatment method for seeds of *Acacia nilotica*. The hot iron stimulated germination from 40% to 84% and is therefore recommended for the laboratory testing of up to a few hundred seeds.

A number of 'bulk' pretreatment methods for larger quantities of seeds were tested. The *A. nilotica* seed lot used was too hard-coated to respond to boiling/hot water. Relatively harsh grinding, macerating or crushing equipment yielded rapid though unpredictable results, often killing the seeds with the thinnest coats. Mechanical scarification in a rotating drum lined with abrasive paper took much longer to accomplish, but was highly effective and offered two further advantages - the progress of pretreatment could be monitored by following weight losses and none of the viable seeds were killed.

Chipping individual seeds was found to be the easiest and most effective 'small scale' pretreatment method for seeds of *Leucaena leucocephala*. The effectiveness of small scale chipping was compared with 170 water temperature and soak durations as bulk pretreatment methods. Seventeen combinations of boiling/hot water were shown to be as good as chipping, and the use of such a systematic and comprehensive range of pretreatments revealed critical boundaries between beneficial, ineffectual and harmful pretreatments.

Untreated, individually pretreated and bulk pretreated seeds of *A. nilotica* and *L. leucocephala* were germinated at constant temperatures of 10, 15, 20, 25, 30, 35 and 40°C and an alternating 20/30°C. The effects of pretreatment and germination temperature are discussed.

1. INTRODUCTION

In common with most legume species, the intact seeds of many tropical legume trees remain fresh and firm but fail to germinate when incubated under moist conditions. The cause is usually an impermeable seed coat which prevents, or at the very least significantly retards, water uptake. These seeds are said to exhibit coat imposed dormancy or hardseededness

(Barton, 1965). In order to stimulate germination, it is necessary to identify and apply a suitable 'dormancy breakage' procedure. Such a technique is normally applied prior to any germination attempt and is therefore called a pre-(germination) treatment or pretreatment. A successful pretreatment for a hard-coated legume seed must either puncture or degrade the seed coat; or disrupt or dissolve the chemical or cellular plugs which render apertures such as the hilum, micropyle, lens and strophiole impermeable (Cavanagh, 1987). However, pretreatment must not be so harsh as to damage or kill the embryo within.

There is considerable scientific, technical and anecdotal literature on the pretreatment and germination of many hard-coated legume seed. Reviews which specifically relate to multipurpose tropical legume trees include those of Willan (1985) for a number of tropical species; Doran *et al.* (1983) and Cavanagh (1987) for *Acacias*; and Hughes (1993) for *Leucaena* species. However, despite extensive source literature, excellent reviews and a lot of empirical knowledge, there is no consensus of opinion about what constitutes the best method of pretreatment for any species.

This paper reports the findings of two systematic and detailed studies on the pretreatment and germination of one seed lot of *Acacia nilotica* and one seed lot of *Leucaena leucocephala.*

2. MATERIALS AND METHODS

Seeds of *Leucaena leucocephala* (Identity Number 44/88) were obtained from the Forest Management Division of the British Forestry Commission, where they had been stored on behalf of the Oxford Forestry Institute at +2°C and 8% moisture content (fresh weight basis).

Seeds of *Acacia nilotica* were obtained from the National Tree Seed Centre, Morogoro, Tanzania. On arrival they were at 7% moisture content.

Seed pretreatments

Chipping was accomplished using a sharp scalpel to carefully remove approximately 1 mm^2 of testa, at the cotyledon end of the seed to avoid damaging the radicle.

Burning a small hole in an individual seed was accomplished by placing the tip of a hot (*circa* 450°C) soldering iron against the seed coat for a known period of time which varied between species. In the case of *A. nilotica* seeds a quiet popping signified penetration of the seed coat and determined the duration.

Bulk seed pretreatment at different water temperatures was achieved by immersing one volume of seeds (secured in a nylon mesh bag) in 10 volumes of water in a thermostatically controlled water bath. Ten water temperatures at 10°C intervals between 10-100°C were combined with 17 pretreatment durations obtained by successively doubling exposure times between 7.5 seconds and 5.6 days. After pretreatment at different temperatures for different durations, the seeds were drained and surface dried in a controlled environment room at 30°C for 30 minutes.

Bulk seed pretreatment by mechanical scarification was achieved by rotating a cylinder (length x radius, 185 x 76 mm), lined with abrasive paper (Grade P60), around its long axis at 100 rpm.

Seed germination

Four replicates of 50 seeds were sown on moist filter paper (in some preliminary work, peat and sand, 2:1, vol:vol) and incubated in the dark at a constant temperature of either 10, 15, 20, 25, 30, 35 or 40°C (Gosling, 1988). Germination was also tested at an alternating 20/30°C, the conditions most commonly used in seed testing stations employing International Seed Testing Association (ISTA, 1993) or Association of Official Seed Analysts (AOSA) rules.

Seed germination was assessed at regular intervals over a 42-day period. Seeds were considered germinated when the emerged embryo showed normal development at three times the length of the seed. The assessment of abnormal seedlings and ungerminated seeds as live or dead was according to the ISTA rules (ISTA, 1993).

Mean germination time (MGT) is a common method for expressing germination rate as a single figure. In this study it was calculated using a modification of the formula of Bewley and Black (1994) according to Jones and Gosling (1994). MGT is equivalent to the average time taken for an average seed to germinate.

Statistical analysis

Angular transformation was applied to all percentage data prior to analysis to homogenise variances. The effects of different treatments on MGT and transformed germination capacity were tested by analysis of variance (ANOVA).

A smoothed bivariate spline (SAS, 1990) was used to model the response surface of germination capacities created by pretreating seeds over a range of temperatures for different durations. Contour lines were constructed from the binomial distribution to give 95% confidence that germination capacities in the highest germination zone were not significantly lower than chipping. Surrounding areas correspond firstly to treatment combinations significantly worse than chipping yet significantly better than untreated; secondly, those treatments which were not significantly different from untreated; and finally, those in the lowest germination zone which were significantly worse than untreated.

3. RESULTS AND DISCUSSION

Comparisons of seed, embryo and seed coat weight between *A. nilotica* and *L. leucocephala* are shown in Table 1. These showed that the seed coat of *A. nilotica* formed a slightly larger proportion of total seed weight than the seed coat of *L. leucocephala* and confirmed the indication of most literature that hardseededness was more pronounced in mature seeds of *A. nilotica* than of *L. leucocephala*. It was therefore considered necessary to carry out a few preliminary pretreatment trials to compare the response of the two species to a limited number of candidate pretreatments. The opportunity was also taken to compare filter paper with peat and sand as two alternative germination media.

Table 1. Dry weight of different parts of *Acacia nilotica* and *Leucaena leucocephala* seeds

Species	Seed weight (mg)			Embryo weight (mg)			Seed coat weight (mg)			Average seed coat weight as % of seed
	min.	max.	mean	min.	max.	mean	min.	max.	mean	
A. nilotica	79.0	241.0	160.8	32.6	104.3	69.3	41.0	134.3	90.5	56
L. leucocephala	39.7	93.0	63.2	16.8	48.7	30.9	20.9	44.3	32.3	51

The conclusions from these preliminary trials were that:-

1. A hot soldering iron was the best method for pretreating small numbers of the harder, slightly thicker seed coated *A. nilotica* seeds. A quiet 'pop' signified penetration of the seed coat of this species and hence when to terminate pretreatment.

2. Chipping with a scalpel was the easiest, quickest and most effective method of pretreating small numbers of *L. leucocephala* seeds. A hot soldering iron was equally effective but controlling the pretreatment time was difficult since there was no audible indication that the seed coat had been breached.

3. An electric coffee grinder used for 5, 15 or 30 seconds was relatively effective at overcoming seed-coat dormancy of a significant proportion of seeds. However, it completely macerated and therefore killed an increasing number of individuals with time. The technique was therefore eliminated from later work.

4. Hot corn oil at 177°C for 7.5, 15 and 30 seconds was used to investigate the possibility that a non-aqueous solvent at a temperature higher than boiling water may be beneficial to seed germination. However, even the shortest exposure time to corn oil proved fatal to all seeds and the technique was eliminated from later work.

5. Boiling/hot/tepid/cold water was chosen as the most promising method of bulk pretreatment for *L. leucocephala*.

6. Mechanical scarification in a rotating tin lined with abrasive paper was chosen as the most promising method of bulk pretreatment for *A. nilotica*.

7. Pretreated seeds of both species ultimately achieved the same maximum percentage germination whether incubated on top of moist filter paper or in a peat and sand medium. However, seed germination was quicker in peat and sand. Nevertheless, filter paper was chosen as the preferred substrate for the main experiments because ungerminated seeds could not be easily recovered or accurately identified as empty, dead or alive from peat and sand - and this was considered of overriding importance to determine whether seed dormancy had been overcome or not.

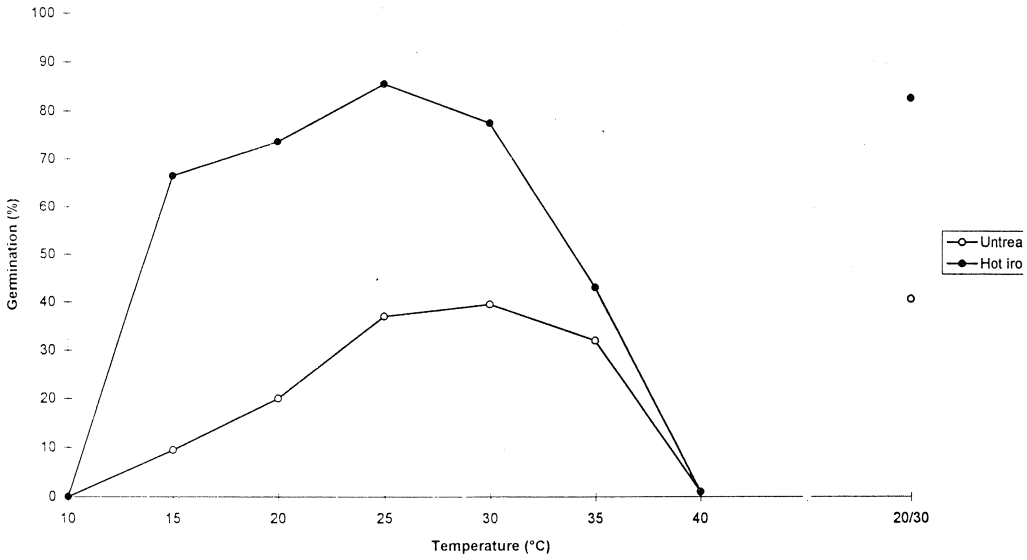


Figure 1. Germination capacity at different temperatures of untreated and hot iron pretreated *Acacia nilotica* seeds

A. nilotica - Pretreatment of small seed lots and identification of optimum germination conditions

A hot soldering iron was used as the small-scale pretreatment method for individual seeds of *A. nilotica*. The effect of incubation temperature on the germination of untreated and pretreated seeds was investigated.

Figure 1 shows the germination capacity of untreated and pretreated seeds incubated over a range of constant temperatures, and at an alternating 20/30°C, the conditions most commonly used in seed testing stations employing ISTA and AOSA procedures. It is clear that neither untreated nor pretreated *A. nilotica* seed was able to germinate at 10° or 40°C therefore these temperatures were eliminated from the statistical analysis. Hot iron pretreatment generally stimulated a 2½ fold increase in germination capacity. Statistical analysis showed that the maximal germination capacity was reached at all temperatures studied between 15-30°C inclusive and also at an alternating 20/30°C. The only temperature at which pretreated seed did not achieve maximal germination was 35°C.

Figure 2a-d provides a complete summary of the fate of all seeds at the end of the germination tests. This is particularly useful for identifying whether any combinations of treatment and germination conditions were simply not conducive to germination (and ungerminated seeds remained alive and unharmed, Figure 2c), or whether conditions were positively harmful (and killed seeds, Figure 2d). Figure 2d shows that the decreased germination capacity at 35°C and 40°C was due to a relatively high percentage of dead seeds.

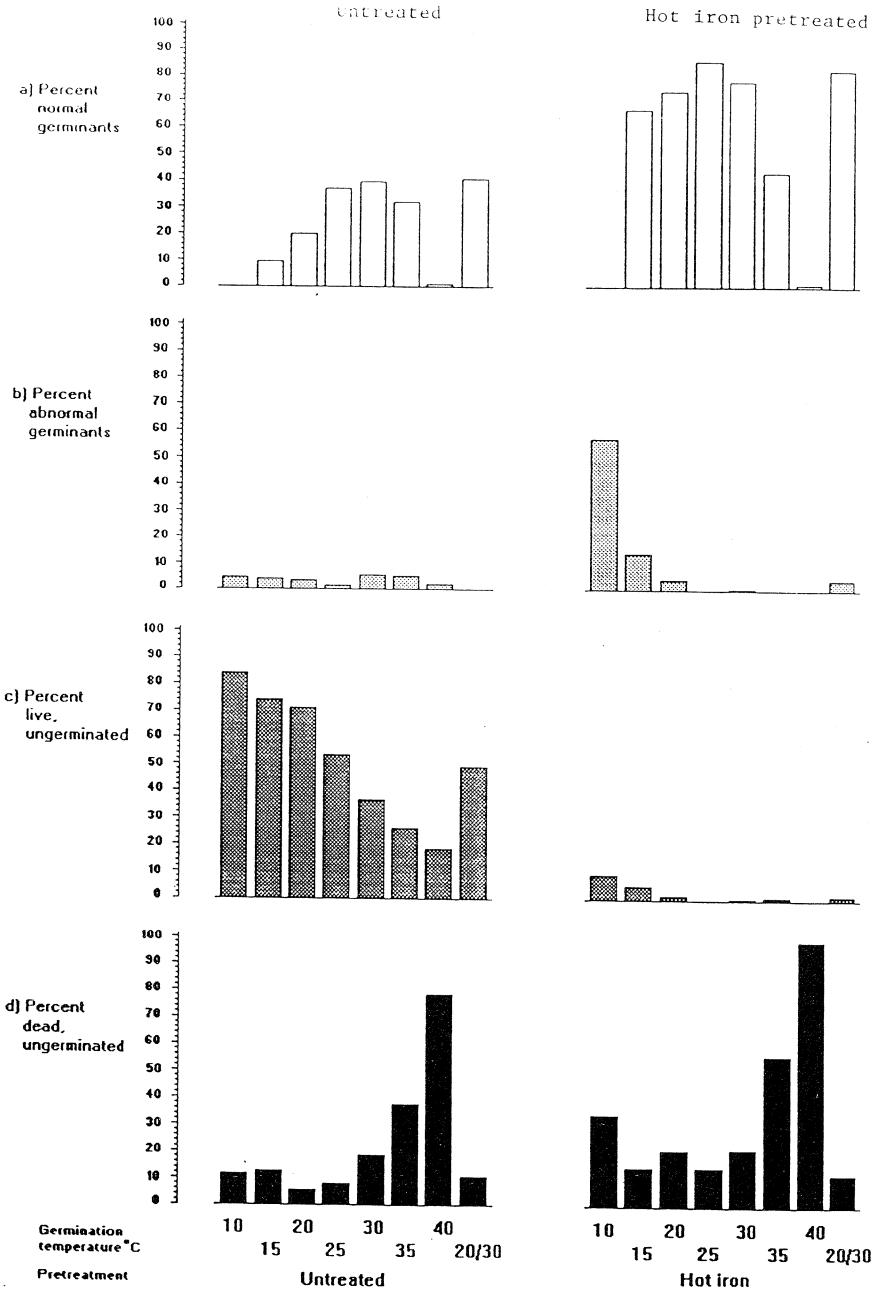


Figure 2. The fate of untreated and hot iron pretreated seeds of *Acacia nilotica* at the end of a 42 day germination test at different temperatures

The above work established that *A. nilotica* seeds had a high germination capacity over a wide range of temperatures and that the alternating germination temperature (20/30°C) prescribed for most tree species by the International Seed Testing Association was appropriate for using as a single set of conditions to evaluate other dormancy breakage pretreatments.

A. nilotica - Bulk pretreatment

Figure 3 shows the decrease in weight of different quantities of *A. nilotica* seeds during mechanical scarification in a rotating cylinder. This clearly demonstrated the ability of the technique to successfully abrade the seed coat and there were several significant advantages of this technique above the harsher coffee-grinder method used for the preliminary trials reported above. First - mechanically scarified seeds reached 92% germination capacity in comparison to coffee ground seeds 78% germination capacity (not shown); second - seeds with the thinnest coats were not killed; third - the progress of abrasion could be easily followed; fourth - as Figure 4 illustrates, the germination of samples removed at different times could be tested so that weight losses from the seed coat could be linked to subsequent germination. In addition, it was found to be possible to repeat mechanical scarification on separate lots, monitor weight loss and predict what the germination capacity of the treated seeds would be. This ability to be able to monitor the progress of pretreatment and accurately forecast subsequent germination is probably a unique achievement within the pretreatment of not only legume species but also any other species.

Figure 5 shows the germination capacity (after a 42 day germination test at different temperatures) of untreated, hot-iron pretreated seeds and seeds mechanically abraded to 85% of the original weight. It is somewhat surprising to note from this graph that the *A. nilotica* seeds responded better to the bulk pretreatment than the hot-iron individual seed pretreatment. Not only was maximal germination capacity reached at all temperatures between 15-35°C inclusive, but some seeds even germinated at 40°C.

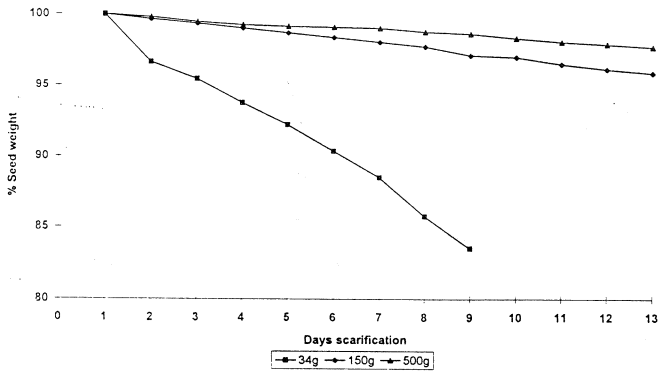


Figure 3. Decrease in seed weight of different quantities of *Acacia nilotica* seeds during mechanical scarification

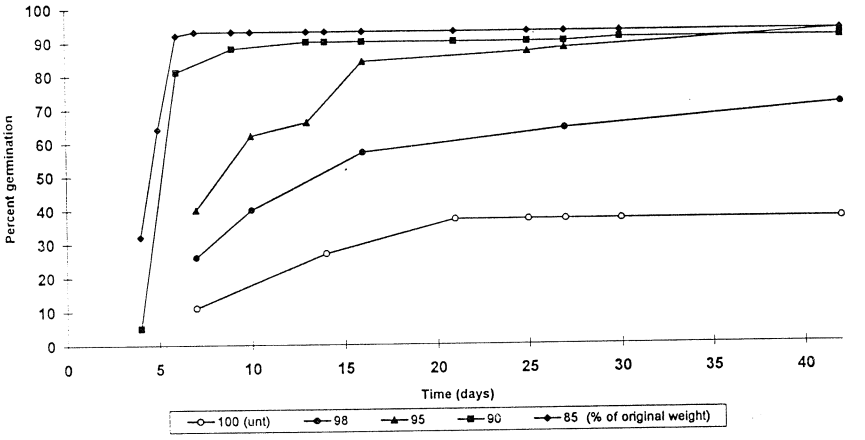


Figure 4. The course of germination at 20/30°C of *Acacia nilotica* seeds reduced to different seed weights by mechanical scarification

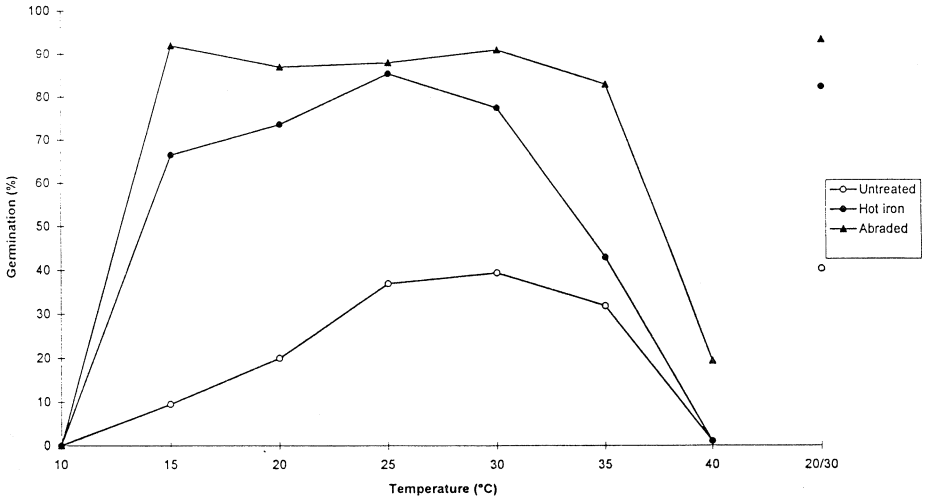


Figure 5. Germination capacity at different temperatures of untreated, hot iron pretreated and mechanically scarified *Acacia nilotica* seeds (reproduced from Gosling et al. 1995)

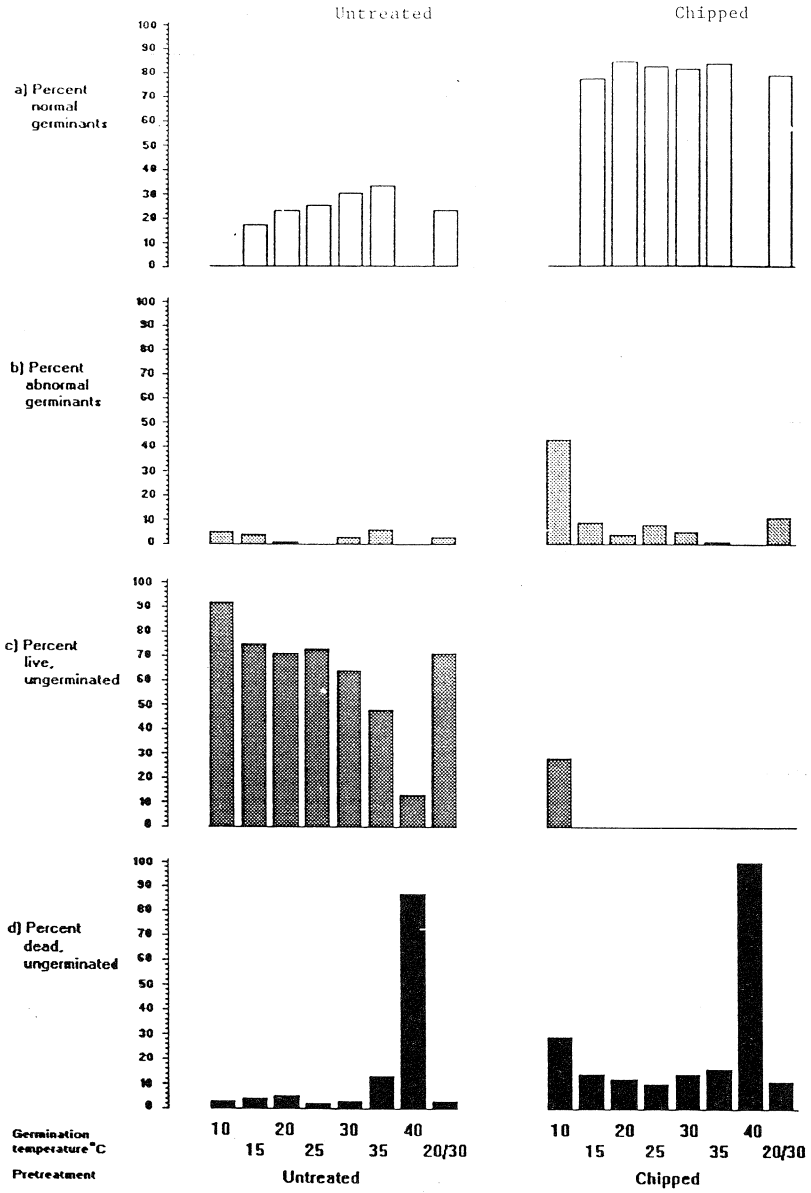


Figure 6. Fate of untreated and chipped seeds of *Leucaena leucocephala* seeds at the end of a 42 day germination test at different temperatures

L. leucocephala - Pretreatment and germination

Figure 6 shows that untreated and pretreated seeds of *L. leucocephala* were even more tolerant of germination temperature than *A. nilotica*. Maximal germination capacity was reached at all temperatures studied between 15-35°C inclusive, and at an alternating 20/30°C.

Figures 7-8 are reproduced from Gosling *et al.* (1995) where they have been more fully discussed. They are included here for comparison with the properties of *A. nilotica.*

Figure 7 is specifically included to show the benefits of employing a comprehensive and systematic array of pretreatment temperature and durations. The germination capacity data resulting from 170 pretreatment temperature/time combinations enabled a smoothed bivariate spline to be used to model the germination response surface shown in Figure 7. This model could be used to not only identify the optimum pretreatment combinations but also to visualise where the boundaries occur between, for example, beneficial and harmful zones. The contour lines in Figure 7 separate four areas of differing germination capacity. The lightest area encloses temperature and pretreatment durations where germination capacity is not significantly different ($p<0.5\%$) from the highest germination capacity stimulated (76-80%). Surrounding darker areas correspond to less beneficial (31-76%), ineffectual (14-30%) and harmful (<14%) treatments.

Clearly there are several combinations of pretreatment temperature and duration which are capable of stimulating the maximum germination percentage possible. For example, at 70°C any duration of treatment between 4 m - 1 h is optimal, at 80°C 30 s - 16 m, at 90°C 7.5 s - 1 m, and at 100°C durations should not exceed 15 s.

Also clear from the lower portion of Figure 7 is that pretreatment temperatures below 35°C never brought about a significant increase in germination capacity, even after more than 5 days soaking, and from the upper right-hand region of the graph any temperature of 50°C and above was potentially harmful if exposed for too long.

Figure 8 shows the germination capacity at different temperatures of four of the best bulk pretreatments (7 s at 100°C, 7 s at 90°C, 30 s at 80°C and 4 m at 70°C) in comparison to untreated and chipped seeds. Although some bulk pretreatments stimulated as high a germination capacity as chipping at several temperatures, bulk pretreated seeds did not germinate as well as chipped seeds at higher germination temperatures and were significantly worse than chipped seeds at 35°C ($p<0.001$). Clearly, even the best hot/boiling water 'bulk' pretreatments were not as good as chipping individual seeds at stimulating high germination over the wide range of germination temperatures investigated. This was in contrast to *A. nilotica* where the mechanical scarification 'bulk' pretreatment was best.

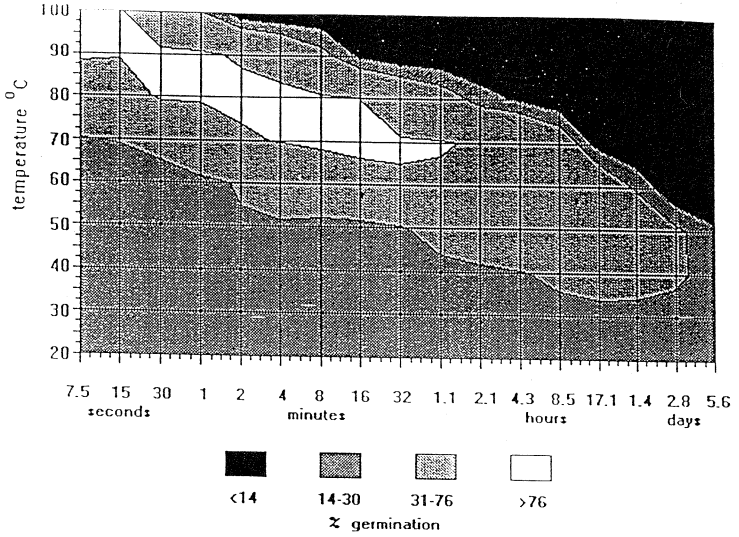


Figure 7. Pretreatment temperature/time combinations which were optimal ☐ ; sub-optimal but significantly beneficial ☐ ; neither beneficial nor harmful ▒ ; or significantly harmful ■ ; to the germination capacity of *Leucaena leucocephala* seeds (reproduced from Gosling et al. 1995)

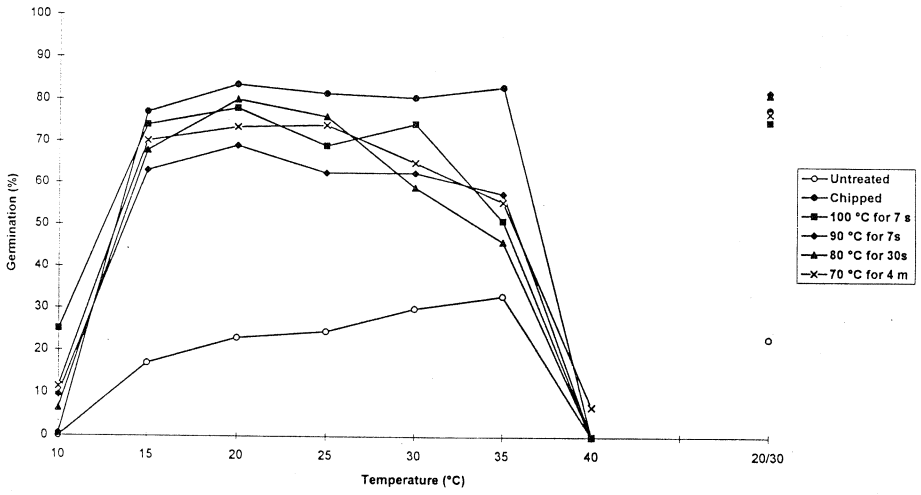


Figure 8. Germination capacity at different temperatures of untreated, chipped and boiling/hot water pretreated *Leucaena leucocephala* seeds (reproduced from Gosling et al. 1995)

4. CONCLUSIONS

Acacia nilotica

A hot soldering iron was the most appropriate method for pretreating individual seeds of *A. nilotica* which had a tougher seed coat than *L. leucocephala.*

Mechanical scarification in a rotating cylinder lined with abrasive paper was an effective and highly reproducible bulk pretreatment method, with the added advantages that the progress of pretreatment could be monitored by following seed weight losses and none of the viable seeds were killed. The disadvantage of the technique using the prototype machine was that it took days to accomplish.

Leucaena leucocephala

Chipping individual seeds with a scalpel was shown to be the easiest and most effective method of pretreating small quantities of *L. leucocephala.*

Several combinations of boiling/hot water temperatures and pretreatment times were as good as chipping at stimulating germination capacity at 20/30°C. The critical boundaries between pretreatments which were beneficial, ineffectual or harmful to seed germination were best presented graphically as in Figure 7. Seed pretreatment methods should be selected from this graph on the basis of time, whether a thermometer is available, and any other local constraints. Consideration should also be given to the fact that even the best boiling/hot water pretreatments did not stimulate such a high germination capacity over such a wide range of conditions as chipping, nor such prompt or uniform germination under any conditions.

5. ACKNOWLEDGEMENTS

We would like to thank Mr Jon Taylor for the majority of the statistical analysis and Mr Andrew Peace for the bivariate spline analysis. Thanks also to Dr Elizabeth Major for carrying out much of the laboratory assessment and data entry, and to Dr Julian Evans for his helpful comments on the manuscript. Finally, thanks go to the British Overseas Development Administration (ODA), Forestry Research Programme for funding project 'R5649 - overcoming seed dormancy in hardseeded tropical legume trees', and supporting the senior author's attendance at this IUFRO symposium.

6. REFERENCES

Barton, L. 1965. Dormancy in seeds imposed by the seed coat. In: Encyclopeadia of Plant Physiology, 2, 727-745.

Bewley, J.D. and Black, M. 1994. Seeds: Physiology of development and germination. 2nd Edition, Plenum Press, New York.

Cavanagh, T. 1987. Germination of hard-seeded species (Order: Fabales). In: Germination of Australian Native Plant Seed. Ed. P. Langkemp. Publ. Inkata Press, 236p.

Doran, J.C., Turnbull, J.W., Boland, D.J. and Gunn, B.V. 1983. In: Handbook on seeds of dry zone acacias. Publ. FAO, Rome, 92p.

Gosling, P.G. 1988. The effect of moist chilling on the subsequent germination of some temperate conifer seeds over a range of temperatures. J. Seed Technology, 12(1), 90-98.

Gosling, P.G., Samuel, Y.K. and Jones, S.K. 1995. A systematic examination of germination temperature; chipping and water temperature/soak duration pretreatments on the seeds of *Leucaena leucocephala*. Seed Sci. & Technol. 23 (2), 521-532.

Hughes, C.E. 1993. Leucaena Genetic Resources: The Oxford Forestry Institute Leucaena seed collections and a synopsis of species characteristics. Publ. Oxford Forestry Institute, Oxford, 117p.

International Seed Testing Association 1993. International Rules for Seed Testing. Seed Sci. & Technol., 21, 1-288.

Jones, S.K. and Gosling, P.G. 1994. 'Target moisture content' prechill overcomes the dormancy of temperate conifer seeds. New Forests, 8, 309-321.

SAS 1990. The G3GRID procedure. Chapter 40, 1311-1330. In: SAS/GRAPH Software, Version 6, First Edition, Volume 2, Cary, NC; SAS Institute Inc. 664p.

Willan, R.L. 1985. In: A guide to forest seed handling - with special reference to the tropics. FAO Forestry Paper 20/2, Publ. FAO, Rome, 379p.

POSSIBILITIES OF MASS PROPAGATION OF MELIA VOLKENSII

B.M. Kamondo[1] and A. Kalanganire[2]

[(1)]Kenya Forestry Research Institute (KEFRI)
Kenya Forestry Seed Centre Subprogramme
P.O. Box 20412 Nairobi, Kenya

[(2)]International Centre for Research in Agroforestry (ICRAF)
Maseno Agroforestry Research Centre
P.O. Box 5199 Kisumu, Kenya

B.M. Kamondo

ABSTRACT

Melia volkensii offers the people in dry areas a possibility of deriving a tangible benefit in tree growing as the tree is fast growing yielding highly prized timber beside the fodder and mulch potential. Prior to 1986, the biggest problem affecting propagation of *Melia volkensii* remained the serious seed dormancy. A seed pretreatment procedure was developed around this time based on scarification of extracted seeds. This has resulted in appreciable increase in *Melia volkensii* planting in the dry areas but its planting programme still falls short of the optimum hectarage. The forest department has expressed desire to grow plantation of *Melia volkensii* and work done at Kenya Forestry Research Institute shows that there are particular proven-ances that can be grown on fairly short rotations.

The main obstacle in the inability to intensify the planting of the tree is inter alia the difficulty associated with seed extraction. Up to 1994, the best option remained in carefully driving the blade of a pocket knife across the stony endocarp and extracting the seed. This yields a few seeds but it is difficult to base large planting programmes on it. Moreover, the subsequent seed pretreatment procedure itself is also tedious. With the development of an extraction method for *Melia volkensii* in 1994 that eased the extraction of seed, mass propagation was made even more possible.

The potential of exploiting this method for mass propagation could be improved if extracted seeds could be stored and used as required. Kenya Forest Seed Centre, in collaboration with ICRAF, engaged in a small-scale low-budget research to investigate the possibility of storing seeds. This paper presents the preliminary findings, which unfortunately were disappointing. However lessons have been learnt that there is still a need to construct a technology that allows for mass propagation of *Melia volkensii* in the nursery. Only then will the seed pretreatment work and now the new extraction method be used in full.

1. INTRODUCTION

1.1 Summary profile of the tree and its potential

Melia volkensii is a fast growing and deep rooted deciduous tree that occurs naturally in semi arid to arid zones of southern Somalia through Kenya and South to Tanzania (Dale and Greenway, 1961). It grows up to 15 meters. The species is the most common tree found on cleared and cultivated land in the drier parts of Embu, and throughout Kitui and Machakos districts in Kenya (Bromley 1994). In these areas, *Melia volkensii* is planted for the production of timber and fuelwood, and for soil reclamation and erosion control. *Melia volkensii* is popular on farms where it is harmoniously intercropped with agricultural crops.

Melia volkensii is a prized species, offering most of the characteristics generally sought for in trees for agroforestry systems. It coppices readily, is fast growing and sheds its leaves in the dry season to provide mulch (Teel, 1985). Its large fruits, twigs and leaves make fodder for goats, cattle and sheep (Milimo, 1986). The timber of the species is valuable, easy to work, durable and strong (Dale and Greenway, 1961). The possibility of growing *Melia volkensii* on a large scale and in plantation for commodity production like sawn timber is a reality that is worth exploring. Provenance trials carried out by Kenya Forestry Research Institute at the coast have indicated that the species can attain a sizeable dbh within ten years (Unpublished KEFRI records).

1.2 Propagation

Although farmers use various methods for propagation of Melia, such as root suckers, the most common method is the use of seeds (Bromley, 1994). Seeds of *Melia volkensii* are dormant and this constitutes a problem for nursery personnel. Milimo (1986), Milimo and Hellum (1990) conducted various germination studies under controlled condition and found that scarified seed germinates within 3-4 days and germination was largely complete in 14 days with the optimum temperature for germination being between 25° and 37°C. In a separate study at Kitui, Nyambati and Konishi (1993) found that the best treatment for breaking seed dormancy is by nipping the caruncle followed by slitting the outer integuments. Although the pretreatment is close to the one recommended by Milimo (1986), one big difference is that the researchers at Kitui did not report on soaking the seeds either before or after slitting as recommended.

Seeds that have gone through the digestive system of livestock are reported to have good germination (Brokensha et al., 1980). The argument advanced is that the animals scarify the seed as it goes through the digestive system. This should raise eyebrows with anyone familiar with the fruit before and after it has gone through the digestive system of the livestock. Although the acids and other corrosive substances found in the digestive system can to a small extent erode the endocarp, it would be impossible for the substances to wear out this heavily lignified endocarp. In fact, the animals regurgitate clean endocarps (which can save the depulping process) but offers no particular basis to expect some pretreatment potential from the digestive system. The good germination associated with animal droppings is most likely from the concentration of the depulped stones as livestock eject the stones while chewing cud in the bomas coupled with natural scarification.

1.3 Conceptual Framework of the Study

The study was motivated by the following facts about the species:

: The species is a favourite tree with farmers and highly rated for growing in the farmlands and has a good potential to be put under plantation in the semi arid areas of Kenya.

: A recommended seed germination method has been available since 1986 (Milimo 1986). Also see appendix 1.

: A new seed extraction method is available. This was an improvement of the previous method based on extraction using a pocket knife (Milimo, 1986, see appendix 1), which, with enough practice, could extract seeds to cope with average seedling demand but would not be suitable for large scale planting. With the new extraction equipment, the possibility of extracting enough seeds to raise nursery stock for the expanded planting scale was made largely feasible.

2. THE STUDY

The study restricted itself to storability of extracted *Melia volkensii* seeds in support to mass propagation.

2.1 Objective of the study

The principle theme of the study is:

Investigation of the possibility for storing extracted seeds of *Melia volkensii.*

2.2 Justification

Currently, *Melia volkensii* is collected in fruit form that consists of a fleshy pulp enclosing a hard endocarp. The recommended stage for fruit collection according to Milimo (1986) is when the fruits have a yellowish green colour. Other people have the opinion that fruit collected after falling yield equally good germination (Nyambati, 1994 pers. commun). The recommended seed handling procedure is to depulp the fruit while it is still fresh, clean the endocarps and extract the seed using a pocket knife (see appendix 1).

If seed is not being used for immediate planting, the current recommendation is that the stony endocarps should be dried to a moisture content of around 8% and stored in the cold room at a temperature of around +3 degrees centigrade (Albrecht, 1993).

Melia volkensii fruit is bulky. Even after extraction, the number of stony endocarp in a kilogram is around 200 stones (KFSC 1992). Storing bulk seed is an expense while in store and in seed distribution when the stones are issued to users. For example, a planting programme requiring 1000 seedlings requires about 20 kilograms of stones that need a 30 l storage container. This is based on one kilo of endocarps (stones) yielding after extraction 100 intact seeds which in turn yield 50 seedlings in the final nursery count. The benefit of issuing

extracted seeds to farmers or/and forest department is then obvious as the same 20 kilos of seed would yield 2000 seeds that can be stored and transported in a kilner jar. While mature and properly dried stones can be stored in air tight containers for several seasons, it is not known under which conditions and for how long extracted seeds can be kept.

Based on preceding argument and the development of the speedier extraction method, it was found desirable to determine how long and under which conditions the extracted seed can be stored. The work was planned to be modest, low budget and there was a lot of optimism that the results would be of great practical value.

2.3 Material and Methods

The method followed the general protocol of storage experiment.

Fruit collection, handling and seed extraction

Fruit was collected from Kitui and Kibwezi in December 1994, following the recommendation that the ripe seeds are recognised by the yellowish green exocarp (Milimo, 1986). Depulping of the fruits was done soon after and the seeds extracted from the endocarps using the pocket knife as detailed in appendix 1.

Seed pretreatment

The seed was divided into 2 lots for each provenance. One lot was used for immediate germination and the other used for the storage process. The germination procedure followed was the pretreatment as recommended by Milimo (1986), i.e nipping to expose the embryo and radicle, soaking in water for 6 hours and then slitting longitudinally through the integument, perisperm and endosperm. (see appendix 1).

Seed germination and storage

The experiment was carried out in the greenhouse, a compromise condition between the controlled lab condition and the uncontrolled nursery conditions. The seeds were sown in 4 replicates of 25 seeds each.

One sample of lot 2 was stored in the cold store at +3°C in air tight containers and another sample of lot 2 was stored on the shelf in air tight containers. The germination experiment was repeated after one month.

3. RESULTS AND DISCUSSION

3.1 Results

Germination results, both before and after storage gave dismal germination rates. In the first trial Kitui provenance yielded 2 seedlings while Kibwezi provenance had 3 seedlings. Only one seedling germinated after storage from Kitui provenance stored in the cold room. Digging up seeds showed that, in both cases, all the remaining seeds rotted.

3.2 Lessons learnt

The experiment, though a failure seen from the practical output expected and the optimistic approach envisaged, has taught a few lessons.

The biggest lesson is that the assumption that the species can tolerate less controlled conditions found in an ordinary glass-house is wrong. It seems that the present conditions need to adapt as much as possible to the controlled conditions that prevail in the laboratory if a reasonable germination is to be achieved.

Even with controlled germination, the sensitivity of Melia to the time of collection should be investigated. The fruit/seed develops in a period of slightly over one year. Over the whole period, there is a length of time in which seed germination can be expected to be zero. This is called the soft fruit stage (Milimo, 1986). At this stage the embryo parts are jelly-like substances. After 8 months, there is a period where all seed parts are fairly well formed. After maturity the fruit falls and the seed can last on the ground for several seasons. This offers a wide range of possible times to collect *Melia volkensii*.

It is important to demonstrate how the germination varies across the various stages when Melia exists as ripe fruit. Even on a branch the fruits do not mature at the same time and a harvester is confronted with a dilemma. If this matter is resolved, then a prescription of harvesting based on factual evaluation will be evolved. Other related problems are:

- Could there be provenance differences in the seed behaviour?
- Is the seed sensitive to the extraction procedure due to the forces at play during extraction?
- Or did the experiment start right away with seeds having inherently low germination?

The fact that laboratory procedure cannot be directly applied to nurseries is a major challenge. The temperature fluctuation seems to be the drawback when *Melia volkensii* seed is sown or plants are planted out in the open. Given that the optimum range is above 27°C, nurseries might have to try to imitate the raising of tea seedlings which is done under a type of airtight polythene 'non-mist propagator' enclosure. Even under this condition, night temperatures might still remain a drawback.

3.3 Recommendations

: Considering the existing potential for *Melia volkensii*, and the existing technology to ease availability of seeds coupled with a seed pretreatment recommendation, there is an urgent need to define one standard technique for handling the seed from collection to the nursery procedure. Although the study should address more factors than the present experiment and with possibility of pushing the agenda out of strict practical research, given the wealth of information and experience existing with different persons, a coordinated effort stands a better chance of quickly finding a solution to the problems of propagating *Melia volkensii*. The optimistic approach motivated by desire to carry out a research with immediate practical value for all its desirability failed requiring academic backup.

: The original objective of addressing storability should be relegated to the second level and tackled after the above problem has been addressed, otherwise the experimental approach should concomitantly address both issues.

4. REFERENCES

Albrecht, J. (ed) 1993. Tree Seed Handbook of Kenya. GTZ Forestry Seed Centre. Muguga, Kenya.

Brokensha D. and Riley, Bernard W. 1980. Mbeere Knowledge of their vegetation and its Relevance to Development. A Case study from Kenya: *In*: Indigenous knowledge systems and development. University Press of America, Inc, Washington U.S.A. pp 111-127

Bromley, T. 1994. Indigenous Agroforestry. *Melia volkensii* in Kenya. Agroforestry Today, Oct-Dec. 1994. ICRAF, Nairobi (Kenya)

Dale, R.I and Greenway, P.J. 1961. Kenya Trees and Shrubs. Nairobi: Buchanan's Kenya Estates Ltd.

KFSC (Kenya Forestry Seed Centre) Seed Catalogue 1992.

Milimo, P.B. 1986. The Control of Germination in *Melia volkensii* seeds. M.Sc. Thesis, 1986. University of Alberta, Faculty of Graduate Studies and Research. Edmonton, Alberta.

Milimo, P.B. and Hellum, A.K. 1990. The influence of temperature on germination of *Melia volkensii*. *In*: Turnbull, J. (Ed.) Tropical Tree Seed Research. Proceedings of an international Workshop held at Gympie, Qld, Australia, 21-24 August 1989. pp 29-31.

Nyambati, R.O and Konishi, H. 1993. Implementation of nursery activities. Project activities. Project working paper. Kenya/Japan Social Forestry Training Project. Pilot Forest Sub-project.

Teel, W. 1985. A Pocket Directory of Trees and Seeds in Kenya. Kenya Energy Non-Governmental Organization, Nairobi Kenya.

Appendix 1.

Recommended seed handling protocol (Milimo 1986)

Fruit Collection

1. Collect mature ripe fruits (yellowish-green) for raising out-planting stock

2. Best germination results were obtained when seeds were placed at temperatures between 30°C and 37°C. Therefore seeds are to be sown in nurseries where mean ground temperature are within this range

Seed extraction

1. Place a dry stony endocarp horizontally on a large cross cut tree stump (about 30 cm in diameter)

2. Place a sharp pocket knife blade midway across the endocarp and gently apply several hammer blows until a crack develops. The knife-blade must not penetrate deeper than the stony endocarp wall, otherwise the seeds will be damaged.

3. Carefully penetrate and open the crack with the sharp edge of the knife-blade by pushing and twisting. The aim is to separate the two stony endocarp halves.

4. Pull out the seeds from their locules in the stony endocarp.

5. Inspect seeds visually and discard those that are mechanically damaged. Seeds without seed coats or with partly removed seed coats will germinate well as long as embryos have not been damaged.

Scarification

1. Break the caruncle off the seed to expose the endosperm at the micropylar end. This will help in estimating the approximate position of the radicle and hence avoid cutting it.

2. Cut longitudinally through integuments, perisperm and endosperm from the centre of the seed to the micropylar end.

3. Soak seeds in water at room temperature for six hours in order to soften the seed coats.

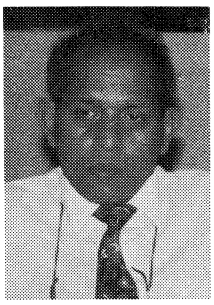
Sowing

1. Place scarified seeds directly into containers filled with germination medium. Keep the medium moist all the time. Visible germination should start by the fifth day after sowing.

TOWARDS THE SETTING UP OF A FOREST TREE SEED CENTRE IN MALAYSIA : POSSIBILITIES AND CONSTRAINTS

B. Krishnapillay, C. Schroeder and M. Marzalina

Forest Research Institute of Malaysia
Kepong, 52109, Kuala Lumpur, Malaysia



B. Krishnapillay

ABSTRACT

This presentation will focus upon the current awareness of the tropical forest and the need and demand for indigenous planting material for the various forest planting programmes in Malaysia.

The paper also discusses the various problems relating to indigenous tree seed procurement and highlights upon a concept for the successful procurement of such planting material on a large scale.

1. INTRODUCTION

The management of natural forest in Malaysia has a long history. The Forest Department of Peninsular Malaysia was established in 1901. Despite the rich natural forest present in the country, the Forest Department was aware of the need for a long term forest management plan. In this plan various activities that included enrichment planting, reforestation and afforestation schemes were incorporated into the silviculture practices to ensure that logged over forest remained in an economically productive state.

The Rio summit discussed in depth the issues concerning tropical forest and there was a general agreement that these forests should be conserved and managed on a sustainable basis. The Malaysian Government recognizes that forest conservation and protection of the environment are important issues and has thus given emphasis to these issues through various Declarations and Acts (Environmental Protection Act 1985, Impact Assessment Order, 1987, Langkawi Declaration for the Protection of the Environment, 1989, Forest principles, 1994). To further show that Malaysia is clearly committed to protect and preserve its natural heritage in perpetuity, there are currently plans to rehabilitate logged over, encroached forests and ex-mining lands with indigenous forest tree species within the country to increase the value of regenerated forest and to reduce the long term pressure on natural forest and also to add to the areas where biodiversity may be conserved. A continuous supply of indigenous planting materials on a large scale is recognised as an essential component of these plans.

2. WHY A SEED PROCUREMENT PROGRAMME?

In the past, indigenous species were not used in the various forest rehabilitation and plantation programmes. Although such seedlings would have high value, problems with irregularity of flowering, poor seed procurement, insufficient knowledge on the timing of seed collection would make the practical methods of collection of the required seed inefficient. A comprehensive seed programme is therefore essential if these problems are to be resolved. Without a good supply of seeds, it would not be possible to carry out tree improvement, establish gene conservation areas or seed production stands and carry out silvicultural trials or the other planting activities mentioned earlier. A number of reports and proposal on this issue are referred (Racz, 1988; Yap, 1989; Schroeder, 1990; Marzalina et al. 1993).

In order to solve this complex problem it was proposed that the Forest Department of Peninsular Malaysia and the Forest Research Institute of Malaysia (FRIM), with the assistance of the German Technical Cooperation (GTZ), develop a mechanism for improving large scale collection, handling and storage of seeds of both indigenous and exotic tree species. In 1994, an official proposal (Krishnapillay et al., 1994), indicating present and expected future demand along with a strategy for the procurement of seeds and planting materials for the whole country was submitted to the Ministry for approval. This was provisionally accepted pending success of its orientation phase which was planned to last two years. At the end of two years the following outputs are expected:

a. Demand and capacity for production of seedlings of indigenous species analyzed and specified
b. Seed production areas in wild stands identified for 7 selected species in a pilot region
c. Phenological observation and reporting techniques improved and operational in the pilot region
d. Trained personnel for seed collection available in the pilot region
e. Selected regional nursery improved/upgraded to handle plant production
f. Concept for the establishment of an advisory and coordinating body formulated
g. Project management system is operational

3. THE PROPOSED SEED CENTRE CONCEPT

The seed centre proposed for this programme is one which embodies a decentralised approach as described below:

a. Seed Centre

The term 'seed centre' would constitute only a coordinating and advisory body whose function would include:

a. identification of the seed collection areas
b. planning and coordination of seed collection
c. networking of nurseries, provision of technical support and supervision of nurseries
d. implementation of seed testing and documentation procedures
e. coordination and dissemination of research results and other relevant information
f. providing training in all relevant components of the programme

b. District Forest Office

On the working level of the programme the district forest offices would play a major role, especially in monitoring and seed collection. The function of the district forest officer with the support of his staff would be:

a. to make regular phenological observations within the seed production areas identified by the advisory unit within the district.
b. to carry out seed collection
c. to transport the seeds to the regional nurseries within the shortest possible time
d. to submit monthly records of phenological observations and quality of seeds collected to the regional nursery and the advisory unit.

c. Regional Nurseries

The proper management and operation of the regional nurseries would be critical for the success of the programme. For Peninsular Malaysia, it has been proposed to set up at least 7-8 regional nurseries. These nurseries would require substantial manpower and infrastructure (seed processing and storage facilities, adequate nursery facilities etc.). They should be strategically located within the states so that seeds collected from the various districts within or from neighbouring states can be brought to these sub-centres within the shortest possible time of about 1-2 days after collection. The proposed functions of these nurseries are:

a. to handle processing and storage/planting of all seeds sent to the nursery in the shortest possible time
b. to link closely with all the related district forest offices
c. to provide raised planting material for planting programmes within the state and in neighbouring states as well
d. to provide monthly records on nursery production and movement of the planting materials to the advisory unit ('Seed Centre')

4. TECHNICAL PROBLEMS RELATING TO SEED PROCUREMENT

Having established the fact that a large scale seed and plant procurement programme is essential if the national plant demand for indigenous species is to be met, the next set of crucial issues to be addressed are the many technical problems related to the tropical seed/plant and their procurement. These are elaborated below. Issues for which solutions have been found are also mentioned.

a. Seeds - Orthodox versus Recalcitrant

Orthodox seeds fall in the category where their seeds when shed from the mother plant have relatively low moisture content (10-12%). On further drying to moisture levels of around 4-5%, these seeds are amenable to storage at 4°C for very long periods without serious loss of viability. Orthodox seeds therefore pose very little problems in storage and handling. They can be collected in large quantities and safely stored and the desired number of plants can be generated from stored seeds as and when it is required for plantation or enrichment programmes.

Recalcitrant seeds on the other hand are those that are shed from the mother plant with high moisture contents (40-70%) and in nature germinate immediately after they are shed. They are intolerant to drying and have relatively high critical moisture levels (20-30%) below which they are killed. They are also temperature sensitive and storage below 16°C generally kills them. Storage studies at FRIM have shown that recalcitrant seeds can be stored for only one week at 16°C and as germinated seedlings at 16°C for a period of 9-12 months. Hence long term storage of whole seeds of recalcitrant species are not possible using conventional storage techniques. Most of the economic tropical timber species, particularly those belonging to the Dipterocarpaceae family produce seeds that are recalcitrant in their storage behaviour. Recalcitrant seeds therefore pose major problems if they have to be used in large quantities for enrichment or plantation programmes. To overcome the problems with these species, there have to be definite planting programmes and proper nurseries where these species can be raised in large numbers when seeding occurs. The planting programmes should be timed according to the occurrence of fruiting.

b. Seed Stands and Seed Orchards

The irregular fruiting habits of many of the tropical species and the sparse occurrence of fruiting trees in their natural habitat is a deterrent to timely collection of these seeds. Collection of enough seeds for large scale use may require so much time that it may become cost ineffective. The solution to this problem will be to set up seed stands or orchards where, through the elimination of undesirable trees, quality seeds in abundance can be produced on a long term basis.

c. Seasonality

For most of the exotic species, fruiting is regular and occurs during specific periods of the year. This facilitates the arrangement for collection, processing and storage. For indigenous species this is not so. While some of the non-dipterocarps like *Endopsermum malaccense* and *Dyera costulata* tend to flower once a year or once every two years, the dipteropcarps, are irregular seed bearers. Generally, they tend to fruit once every 3-5 years and in some extreme cases once every 6-7 years. However, from the 18 years of phenological records collected by FRIM from all over the country, it has been observed that similar species tend to flower and set seeds during different years in the different states. This observation leads one to believe that every year some trees of different species are fruiting in one forest reserve or another. Hence, through a systematic and thorough phenological observation procedure, a good amount of seeds of dipterocarps can be collected somewhere in most years.

d. Mast Fruiting

This is a phenomenon that occurs once in six or seven years. During mast flowering and fruiting, almost all the canopy (trees) species in the forest flower and fruit simultaneously. This has remained a mystery until recently and a number of environmental cues were suggested as possible triggers. They include temperature, prolonged drought and increased sunlight hours. So far the best explanation appears to be the correlation between mast flowering and a drop in night temperatures of about 2°C below the minimum average over a period of three to four consecutive days (Ashton et al., 1988). During such a mast fruiting season there is an abundant supply of seeds of various species especially those of dipterocarps. By the proper deployment of work force, a very large quantity of good quality seeds of the desired species can be collected.

e. Monitoring and Phenology

Monitoring and phenological observations are very critical and essential for successful procurement of seeds of indigenous species. As many of the species are irregular seed bearers, a good monitoring system is essential to detect, if trees are flowering or fruiting. At present, the monitoring of flowering and fruiting is carried out on an ad hoc basis.

f. Collection and Distribution

Current methods of collection of seeds and planting material of indigenous species include ground seed collection and the collection of wildings. In most cases the seeds collected from the ground are heavily infested with insects and fungi. It is therefore essential to have a properly organised system for collection and distribution of seeds.

g. Seed Handling

Seed handling includes transport, processing, cleaning, drying and testing of seeds after collection and before storage. While these processes are relatively simple and standardized for orthodox seeds, the same cannot be said for indigenous recalcitrant seeds.

Recalcitrant seeds have to be carefully handled right from the point of collection. Transporting of seeds should be done in bags with the seeds loosely packed and they should be transported to the nursery under cool conditions to prevent overheating. Once at the nursery, the seeds should be processed and sown on nursery beds within one to two days of collection. Delay or negligence at any of these stages would result in reduced rate of germination. At present, although the protocol for handling recalcitrant seeds has been worked out at a laboratory scale, this has yet to be extended as a large seed handling programme for the simple reason that there has to date been no real demand for planting indigenous species on a large scale.

h. Storage

While orthodox seeds (mainly of exotic species) can be stored safely after drying to a moisture content of 4-5%, the recalcitrant seeds (mostly indigenous) cannot. These seeds cannot be dried and stored and hence have to be planted immediately on receipt at the nursery. Research has shown that these recalcitrant seeds can be kept as whole seeds only for a period of 1 week at 16°C. If they are germinated, then they can be maintained up to a period of 8-9 months at 16°C under subdued light conditions.

i. Seed Research and Testing

At present the Forest Department does not have the facilities or manpower to carry out seed research and testing. These tasks have been undertaken by FRIM, which maintains a modern seed laboratory that carries out research in the following areas:

a. seed germination physiology
b. seed storage trials
c. seed treatment trials
d. seed testing and certification
e. seedling storage trials

After 15 years of studies, it has been amply demonstrated that the seeds of many of the forest species cannot be stored using conventional storage methods. High technology research methods are now being exploited to store the excised embryos of these seeds, however, these techniques are, for the time being, expensive for normal use.

j. Vegetative Propagation

Research on the vegetative propagation of indigenous species is part of FRIM's research programme. A programme to this effect which has been initiated under the Malaysian-German Forestry Research Project (GTZ) is already in place at FRIM. To date about 12 species of dipterocarps and two non-dipterocarps have shown potential for propagation by cuttings. Two of these species namely *Endopsermum malaccense* and *Hopea odorata*, are now being tested for large scale production.

To date vegetatively propagated indigenous species have not been used for planting either in the natural forest or in the forest plantation programmes. It would appear that it would be some years before planting stocks from cuttings for large scale planting operations would become available.

5. ISSUES TO BE RESOLVED TO ACHIEVE OBJECTIVES OF THE PROGRAMME

The following are some of the major tasks that need to be addressed in order to achieve the overall goal of sustainable use of forest resources in Peninsular Malaysia and to achieve the project purpose which is the ensuring of sufficient planting stock of indigenous species of good quality available for the various planting programmes in the future.

1. Seed Sources

There is a need to select, establish, manage and conserve good seed sources. This is a process that should start with the evaluation of each good seed source so far utilized and should proceed to search for possible new sources. At present, there exist some good seed sources for exotic species but none for indigenous species. One of the urgent tasks of the seed and plant procurement programme will be to identify such seed sources. To effectively resolve this task, there is a need for close cooperation with the various district forest offices who are acquainted with the forest under their districts.

2. Monitoring of Flowering and Fruiting

As the tropical indigenous species are irregular seed bearers and flowering could occur over different years at different locations for different species, it is pertinent that a systematic monitoring procedure should be designed and implemented in each forest district. This would involve the training of personnel (possibly forest rangers) to recognise flushing, flower initiation, fruiting and stage of maturity of the seeds for collection. The trained forest personnel should be instructed to carry out observation rounds on a monthly basis of the forest within their districts and report their findings in a prepared format at the end of each month, to a coordinating body which is designated as the Seed Centre.

3. Tree Climbing and Seed Collection

Seed collection off the ground (though widely practised) has many problems. Generally, seeds that fall on the ground are attacked by insects or fungi or tend to germinate rapidly. In many cases 50% of the seeds brought back to the nursery from a ground collection die due to the damage that occurs when they fall to the ground. To ensure that good quality seeds of the right stage of ripeness are collected, it is inevitable that the collection must be made from the tree. As the fruit setting is dominant and the emergent indigenous trees are very tall (40-60 metres in height), there is a need to train tree climbers to do this collection. Experience (in FRIM) has shown that young energetic staff could be trained to climb the trees using special climbing equipment. Such a training would take about 2-3 weeks before a climber becomes familiar with the equipment and master the methods of ascending a tall tree, working in the crown of the tree and later coming down once the task is accomplished. In this programme, tree climbing training is essential for its success.

4. Seed Procurement

The timing for seed collection, the handling of the seeds from collection to distribution, a reliable dispatch system and sufficient standardized methods developed are all important to the success of the programme. For some exotic species, it is often necessary to purchase and import the seeds. The seed centre, therefore needs the capacity to deal internationally with the commercial seed trade for the purchase of large quantities of seeds or to negotiate exchange arrangements with specialized agencies for research quantities. Such mechanism is currently lacking and needs to be established.

To broaden the genetic base of some of the exotic species currently planted, there may be a need to carry out organised seed collection mission to the centre of origin of such species to procure good germplasm for the breeding programmes.

5. Recalcitrant Nature of the Seeds.

Recalcitrant seeds have poor storage qualities. Because of this and their sensitivity to the environmental conditions, clear guidelines on their handling need to be formulated and standardized for use in all the regional nurseries. This would involve methods of collection, packing, transport, processing, nursery techniques and the raising of healthy seedlings. Knowledge of many of these methods are available at FRIM only at laboratory scale and need to be tested for utilization in large scale collections.

6. Seed Handling and Seed Storage

In order to safely and effectively store large quantities of seeds until they are required for distribution and sowing, there is a need to have storage facilities in the form of cold rooms at different temperatures. Such facilities should be made available in the programme. It would be appropriate to have at least 4 temperature regimes (below 0°C, 4°C, 8°C and 16°C) for research and storage purposes. It is also advisable to have a truck-borne cold container for each nursery to bring back seeds from distant places where travel time could be more than 2-3 days.

7. Nurseries

To be able to handle large quantities of seeds and for raising seedlings (in the region of 4-5 million seedlings per year per nursery) to meet the planting demands of the forest programmes, it is very important to upgrade some nurseries. At present the existing nurseries have only the very basic infrastructure and equipment. It is proposed that some nurseries in the country be upgraded where production levels envisaged in this programme could be achieved. An alternative to this could be to have the collected seeds contracted out to private nurseries for raising the seedlings.

8. Rehabilitating Programmes

To rehabilitate the logged over forest, encroached lands, road side and ex-mining areas, a large number of seedlings (in the region of 10-15 million) will have to be planted annually. This would mean a large manpower involvement, if implementation is to be successful. To solve this problem it may be advisable to carry out such activities through private contractors under the supervision of the Forest Department staff.

9. Seed Testing

Seed testing is aimed at typifying seed lots so that the purity and germinative capacity of each seed lot is authoritatively labelled. Such information is useful for both growers and suppliers. The advantages of seed testing carried out in this programme include:

- better communication with the growers
- early identification of problem seedlots, and
- a flexibility to run tests and do research when required.

It is therefore necessary and important to incorporate a seed testing laboratory within the programme.

The essence of good seed testing is the application of reliable standard methods of examination to ensure that uniform and reproducible results are obtained. These determinations are best made by adherence to International Rules for Seed Testing or by preparing similar prescriptions for species commonly handled by the programme.

10. Seed Documentation

It is the responsibility of the seed and plant procurement programme to make and keep, in easily accessible form, full records of the movement of seed lots/seedlings in and out of the store and nurseries. Essential data such as seed lot number, species name, number of parent trees, weight of sample, origin details and viability should accompany each consignment. These data will place a grower in a better position to identify superior seedlots or plant materials when seeds or plants are required. It will allow for the elimination of inferior lots as the programme progresses. Such a system is clearly lacking and needs to be instituted.

11. Seed Certification

Seed certification has been defined as the guaranteeing by the duly accredited agency of the provenance, purity, quality, clean condition, etc. of a given lot of seeds. The main certificates usually prepared include the phytosanitary certificates, seed quality certificate and seed origin certificates. The programme will provide useful information and documentation for this

certification, but the certification itself will be the responsibility of independent officially accredited organizations. Such a programme for forest seeds are not in effect and need to be instituted to ensure quality planting material in the future.

12. Research and Development

Research aimed at optimising methods for collection, processing, storing and germinating forest seeds collected on a large scale is an essential function of the programme so that seed longevity and germinative capacities are maximised.

The seed and plant procurement programme should take up research aimed at optimising seed collection strategies, promoting measures to conserve the genetic resources of important species; studying genetic variation and breeding systems; carrying out species and provenance trials and establishing seed orchards/stands. The last item, however, is usually best undertaken in close cooperation with the tree improvement unit. The mutual benefits of this cooperation to the programme includes continuing access to the latest information on which seed is 'best'. The benefit for the tree improvement specialist is an assurance that the product of their research is collected, stored and distributed efficiently.

The ability of this programme to undertake research work will depend on the level of staffing and the physical facilities available to it. It is important that these factors be given due consideration when plans for the programme are being drafted.

13. Cooperation, Communication and Networking between Agencies

For the successful functioning of a seed procurement programme, there is an important need for a strong cooperation and networking between the relevant agencies involved in the programme. The issues of priority that need to be resolved include the following:

- there has to be a consensus on the indigenous species that will be used in the plantation programme
- there is a need to build up the relevant knowledge on the species to be planted. FRIM is currently involved in this aspect. However, efforts need to be stepped up to provide such information in the shortest possible time.

6. IMPORTANT ASSUMPTIONS AND COMMITMENTS FOR SUCCESS OF PROGRAMME

6.1 Important Assumptions

The main concept of this proposed project is to make available sufficient quantity of good quality planting stock of indigenous species. To ensure this the following are some of the important assumptions that are critical for the success of the project:

- policy continues to stress on enrichment planting with indigenous species
- funds for planting programmes available for enrichment planting, underplanting of existing plantations
- participating organisations continue to cooperate and exchange relevant information
- regular fruiting of the important species

- sufficient manpower available for seed collection
- private investors are interested in nurseries
- the participating institutions especially the Malaysian Government stay in favour of private sector involvement
- relevant ministry ensures the enforcement of the seed regulations
- institutions involved in the seed procurement programme cooperate
- trained persons stay in their functions (at least for two years)

6.2 Extension and Training

It is of little value if a successful tree seed and plant procurement programme optimizes seed quality but, due to lack of information and knowledge, the grower makes poor use of the seed. Thus an effective extension and coordinating service is essential for successful implementation of the programme. This service is necessary to optimise the use of available information. The programme should therefore also provide the following services to ensure the optimum use of the seeds:

- advise the states on the selection of the most appropriate species and provenances to meet their planting programmes
- provide information on seed collection techniques, the physical and physiological characteristics of seed (e.g. maturity indices) and propagation techniques
- collect and disseminate data on the ecology of individual species, thus providing a basis for an improved understanding of the species habitat
- disseminate information through appropriate symposia, study tour and publications
- prepare directories as well as regular newsletters of seed availability in the various states
- encourage quarantine practices which minimize the risk of harmful insects and diseases being spread by the seed lots being distributed.

7. CONCLUSION

The current trends show that the demands for high quality planting material of both indigenous and exotic species will continue to increase in future. By the year 2000, around 10-30 million seedling are expected to be required annually to meet planting demand. The current ad hoc method of procuring planting material will not be able to meet such demands. Unless the imbalance between demand and supply is corrected, the implications on forest production and function cannot be adequately met. The need therefore for a quantum leap in seed supply is obvious and the urgency plain. The strategic mission ahead therefore to correct the supply systems is through the setting up of a comprehensive and integrated seed procurement programme to undertake the following tasks.

a. translation of research findings into easy guidelines for field workers in seed technology and management
b. selection, establishment, management and conservation of seed sources
c. phenological monitoring and observation
d. improved skill levels in seed collection, handling, storage, transportation and testing
e. documentation and testing of seeds
f. upgrading and rehabilitation of nurseries

It is proposed that under the seed and plant procurement programme, a coordinating and advising body provisionally termed as the 'Seed Centre' be formed to coordinate the effective functioning of the tasks outlined above.

8. REFERENCES

Ashton, P.S., Givinish, T.J. and Appanah, S. 1988. Staggered Flowering in the *Dipterocarpaceae*: New Insights into Floral Induction and the Evolution of Mast Fruiting in the Aseasonal Tropics. American Naturalist 132: 44-66

Krishnapillay, B., Kuhmann, A.S., Yahaya Mahmood, Sashiah Abdul Karim, Abdul Khalim, Abdul Rasip and Schroeder, C. 1994. Seed and Plant Procurement Programme for Peninsular Malaysia Proposal submitted to the Ministry of Primary Industries, Malaysia. 70 pp (Unpublished)

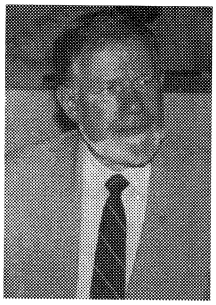
Marzalina, M., Krishnapillay, B. and Yap, S.K. 1993. Problems and Solutions in Collection of Tropical Rain Forest Species. Paper Presented at the Conference on Genetic Conservation and Production of Tropical Forest Tree Seeds. Chaingmai, Thailand.

Racz, J. 1988. Report on Current Status of Seed Management in FRIM. GTZ Report, FRIM, Malaysia.

Schroeder, C. 1990. Report of Seed Supply for Malaysia. Malaysian-German Forestry Research Project. Project Report, FRIM, Malaysia.

Yap, S.K. 1989. Role and Function of FRIM's Seed Centre in the Production and Management of Improved Seeds. Proceedings of the GTZ Regional Seminar, FRIM, Malaysia.

SEED PROCESSING - EFFECT ON SEED QUALITY



E.B. Lauridsen

Danida Forest Seed Centre, Krogerupvej 3 A
3050 Humlebaek, Denmark

ABSTRACT

Seed quality encompasses physical, physiological and genetic aspects. In traditional seed processing operations efforts are made to maximize the physical and physiological quality of seedlots. There is evidence that these efforts may affect the genetic quality of the seedlot.

Morphological, physical and physiological characteristics of seed are the result of the effects of genes of the seed mother tree, environmental conditions at location of seed mother tree, and the interaction of the two. The characteristics of the progeny on the other hand would ultimately be regulated by maternal and foreign paternal gene effects interacting with the effect of the environmental conditions at the planting location of the progeny. Characteristics of the mature seed may therefore not necessarily be correlated with the characteristics of the resultant tree and its products.

Maximizing seed lot quality by maximizing only physical and physiological qualities, may therefore mean that progeny quality is not maximized.

The selection for special seed characteristics during processing operations - intentionally through grading, or unintentionally through damage of seeds during handling operations - may remove the seed of whole families, or part of families. Because seed and progeny characteristics are independent, then the systematic selection of different seed characteristics may cause a random selection of progeny characteristics, i.e. families of good progeny characteristics as well as families of poor progeny characteristics may be removed.

The effect of selection may be of no importance where the seedlots include a broad genetic variation. However, for various reasons, during seed collection operations the sampling of genetic variation may be incomplete. In addition, seed collection for genetic conservation is often done in populations of only few individuals. By neglecting the genetic aspect in seed handling operations there is a risk that efforts to capture specific genetic variation from natural sources, plantations, or improved stands would be counteracted.

The paper discusses all three aspects. It is suggested to intensify research work on the genetic aspect in order to obtain more knowledge of the effect of seed processing operations on the genetic variation in seedlots, and the implications of any changes on the work of genetic resource conservation and genetic improvement.

1. INTRODUCTION

The present symposium has the title: Innovations in seed technology. As a matter of fact, possibly we ought to apply more emphasis on the proper utilization of existing knowledge and technology rather than looking for new technologies. Having done that, innovations in seed technology would then be appropriate.

The techniques and procedures of seed operations are closely linked to the biological systems of the particular seed. There is a substantial amount of knowledge existing on the techniques of seed handling (equipment and operation) and on seed biology.

To understand how seed quality is affected by seed operations is to understand principles of genetics and seed biology. In seed supply operations, the best results can be obtained when we utilize our knowledge of these principles efficiently.

The present paper goes through the basic principles of seed processing and their possible effect on seed quality.

2. SEED QUALITY

Seeds are living systems and their quality is influenced much by environmental conditions during development, harvest, processing, transport and storage.

Quality includes physical, physiological, as well as genetic aspects.

The physiological and physical quality of a seed has to do with its viability and vigour, its health, the condition of the morphological structures, as well as the specific gravity of the seed. Seed health concerns not only pests and diseases which directly affect the viability and vigour of the seed, but also those that may cause problems if transferred along with the seed to new environments, i.e. seed borne diseases.

The physiological and physical quality of a seed population (seed lot) is the sum of the physiological and physical qualities of the individual seeds in the population. In addition comes any impurities in the seedlot.

The genetic quality of a viable seed population has two aspects: one which is the genetic superiority of the mother tree population (the provenance) as compared to other provenances, which is potentially inherited in the seed population, and another which refers to the degree to which the genetic composition of the mother tree populaton is represented in the seed population.

The total quality of a seedlot is expressed in its ability to be stored, to germinate and produce usable plants, together with the degree to which the genes in the seed embryo population represent the genes in the mother tree population. A weighted index for total quality may be used to demonstrate the importance of the genetic variation. The weights are economic values of each quality aspect.

It is assumed that seed quality is maximum at seed maturity, although there are many exceptions to this. Having reached maximum, seed quality decreases with progressing time

due to normal ageing of live material together with the effect of the prevailing environmental conditions, including collection, handling and storage operations. The rate of deterioration depends on the type of seed, the environmental conditions and the protection afforded the seeds. Most important in this connection: Whenever we manipulate with a seedlot, we are manipulating with its quality!

3. SEED MORPHOLOGICAL AND GENETIC STRUCTURES

A small repetition of the morphology and genetic make-up of seeds will facilitate the presentation of the subject: damage to quality.

In the following the term "seed" includes both fruits and true seeds.

A developed, true seed has developed from the maternal ovule normally following fertilization by a foreign male gamete (outcrossing). It includes the seed coat, the endosperm in angiosperm seeds or the mega-gametophyte in coniferous species, and normally the embryo, the coming plant. The seed coat has different forms and structures in different plant species, specifically in respect of thickness, as well as different functions; dormancy for example is often located in the seed coat. The endosperm may occupy a good part of the seed (*Rhododendron spp., Sambucus spp., Ribes spp.* for example), or it may be rudimentary, its function as a nutrient store having been taken over by the cotyledons (many leguminous seeds). The mega-gametophyte in most coniferous species occupies the larger part of the seed.

The true fruit develops from the maternal mega-sporophyll, which in angiosperms is also called carpel. In angiosperms one or more carpels will form the ovary containing the ovule(s), which again develops into the true fruit structures. They are in angiosperms the pericarp, including the exo-, meso-, and endocarp, and the seed. Several mega-sporophylls on a central axis form the coniferous cone.

There are three different sources of genetic material (DNA) in plants: nuclear, mitochondrial and chloroplast. The first is inherited and segregating according to mendelian laws, whereas the two last ones are not (Wang, 1992).

In the seed, the embryo is normally diploid following fertilization of the egg with the male gamete. The father and the mother contribute thus genetically each with one set of chromosomes or nuclear DNA. In addition to this, however, the mother and father contribute to the embryo with cytoplasm containing organelles which have their own independent genetic systems; particularly the mitochondria and plastids. The situation is reviewed by El-Kassaby et al., 1992. Chloroplast DNA in angiosperms is usually inherited maternally, whereas in coniferous species it is inherited predominantly paternally (Wang, 1992).

The mega-gametophyte in coniferous seeds is maternal haploid, and the endosperm in angiosperms is multiploid originating from fusion of some of the female nuclei and one of the male gametes.

The seed coat in both coniferous and angiosperm seeds is maternally diploid. See figures 1 and 2.

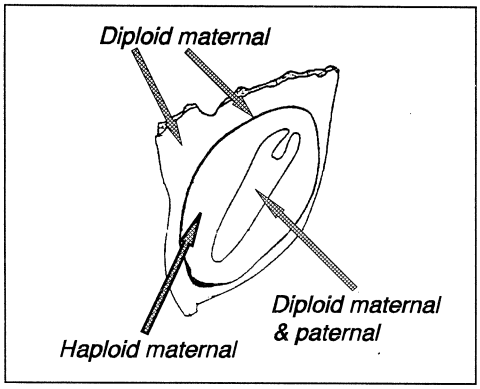


Figure 1 Genetic structure in gymnosperm seed

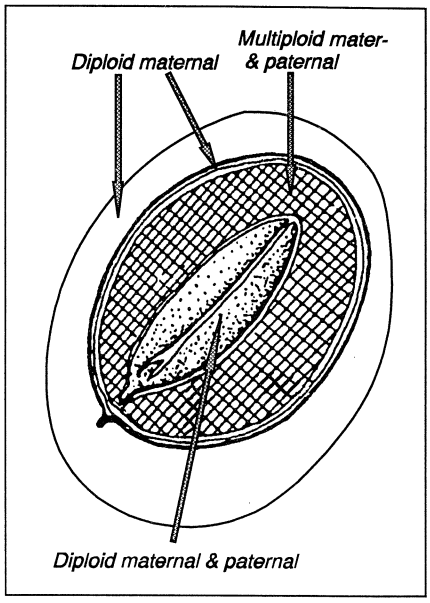


Figure 2 Genetic structure in angiosperm seed

In many important coniferous seeds, where fertilization does not occur until the seed has grown to full size, the most important seed properties have developed under sole maternal and environmental control. In most angiosperm seeds, where the embryo develops concurrently with other structures, the foreign male would undoubtedly have some influence. It is known for example that in *Tectona grandis* selfing gives smaller fruits than crossing. Altogether, the situation is possibly much more complex in angiosperms than in conifers.

The maternal influence during seed development is further aggravated since the fruit structures - including the cone in coniferous species - are possibly developing under sole maternal control, although the male pollen has the important function to trigger the start of the development of the fruit or cone. Fruit parts are in some angiosperm species responsible for important dormancy functions.

The maternal genetic system may thus be assumed to dominate significantly in the development of some important structures and functions of fruits and seeds responsible for the germination behaviour of the seeds. Knowledge about this situation has gradually accumulated.

In addition, and interacting with the maternal genetic effects, seed characteristics are also very much affected by the environmental factors prevailing during seed development, and may also be affected by position of the inflorescence on the mother tree, or the position of the seeds in the inflorescence or cone. Fenner 1992 has revised this complex.

The performance of the seed progeny in its ultimate growth locality is influenced by the diploid genotype interacting with the environmental conditions. The non-nuclear genetic systems may also be important for plant development and health.

Thus, the development of seed characteristics is regulated by genetic and environmental effects that would be different from those regulating the development of the embryo into the mature plant.

Handling seeds in various ways selects, directly or indirectly, various shapes, sizes or types of seed to remain in the seed bulk to be sown. This selection may change the frequencies of genes represented in the embryos of the seeds. It would logically follow to examine the influence which seed handling operations would have on the frequency distribution of genes represented in the embryos.

The effect of handling operations is normally verified only by examination of germination and early seedling development. This will not provide information on any changes that may have occurred to the genetic quality. Specific studies are necessary to reveal such changes.

4. SEED PROCESSING: TERMINOLOGY & CONCEPTS

The term "seed processing" has slightly different meanings in relation to agricultural seeds and in relation to tree seeds in forestry; further, in forestry the term is not consistently used.

Crop seeds and tree seeds are now handled and used increasingly by the same people. Forest seed centres may provide seed for agro-forestry programmes. Agro-forestry programmes procure both kinds of seed, and villagers are hopefully using more and more tree seed, which they may often have to procure themselves.

In agriculture, processing includes cleaning, drying, seed treatment (pesticides), packaging, and storage. Cleaning again includes scalping, hulling, shelling, cleaning, and up-grading (Thomson 1979). Threshing is considered part of the harvesting operation, i.e the removal of the seeds from the plants, but the removal of the hulls of leguminous fruits would also be termed threshing.

In forestry, seed processing operations are sometimes considered to include the steps from the time the fruits, cones or seeds are received at the processing plant and until the seeds have been made ready for sowing (Edwards 1979). This terminology is similar to Thomson's. It includes storage operations.

Sometimes, operations after harvest are divided into i) handling prior to processing (Stubsgaard 1989) and ii) seed processing (Stubsgaard and Moestrup 1991; Willan 1985). The former operations are generally carried out in the field - after collection, but before the seeds have reached the seed station. However, they are in fact also processing operations. They may include pre-curing, cleaning, extraction, drying and temporary storage. Species and local conditions in terms of distance, environment, and availability of resources, rather than the nature of the operations, would determine whether some operations would have to be carried out already in the field. The following will therefore not distinguish between in-field and in-station operations.

Traditionally, seed processing may be understood to be all operations after harvest that aim at maximizing seed viability, vigour and health. The operations should also ensure that specific requirements for seed uniformity in one or more morphological characteristics, and

in germination rate are adhered to. The purpose of such uniformity is to minimize costs of any further processing and nursery operations.

In detail the purposes are:

- to lower cost of further processing and storage, including transport. This is achieved by reducing the bulk of the seedlot by cleaning for debris, and by removing empty, or fractured seeds,

- to bring all seeds to optimal maturity; this is achieved by after-ripening,

- to free seeds of fruit structures; this is achieved by extraction,

- to increase the longevity of seeds, and to lower the risk of transfer of diseases or pests between countries; this is achieved by removing material that may harbour diseases and pests,

- to increase longevity of seeds; this is achieved by drying seeds to a moisture content where fungi and insects, responsible for seed deterioration, are not active,

- to increase longevity of seeds; this is achieved by treating with fungicides and insecticides that reduce the activity of fungi and insects,

- to improve general seed vigour; this is achieved by invigorating seeds by conditioning,

- to reduce variability in vigour; this is achieved by invigorating, or by removing low vigour seeds,

- to reduce variability in seed shape or size; this is achieved by grading, or by pelleting.

Storage of seed is a specific operation with the objective of maintaining the seed quality as best possible for a given time. Transport is conveniently included here, because the requirements for the maintenance of seed quality are the same in short as in long term storage. Storage is really not a process in itself. Ideally, seeds entering a seed store should come out with unchanged characteristics. In this connection, the term "temporary storage in the field" is used in a confusing manner, because it often includes after-ripening or pre-curing and drying. It would be useful to keep temporary storage as a storage operation proper with the above objective, and to keep pre-curing and drying as processing operations with their particular objectives.

Are pre-treatment operations part of seed processing proper? Pretreatment actually means pre-germination-treatment and includes all treatments aiming at alleviating dormancy. It is becoming more and more common to store pretreated seed. Since pretreatment helps to make a uniform germination of seeds, it may be considered part of the processing operations.

Seed treatments include seed dressing with insecticides or fungicides and pelleting. The operations are meant to prepare the seeds to withstand transport and storage, and sometimes to make seeds of a uniform size or shape (pelleting). This operation is therefore also part of processing.

It is obvious that seed processing operations are directed towards the physical and physiological qualities of the seeds. The interesting question is how these operations will affect the genetic structure of the tree population derived from the seeds.

5. SEED PROCESSING OPERATIONS AND SEED QUALITY

It would not be difficult to agree that the seed collection or harvesting step and its planning, are among the operations that are most critical for the resulting seed quality. It may not be possible to rectify flaws in this operation in subsequent operations, viz inadequate sampling of mother trees, too late or too early collection. On the other hand, the good quality of seed from a well performed collection may easily be lost if some important subsequent operations are not done in the optimal way.

Damage to the morphological and physiological structures due to subsequent faulty seed handling is well described, and the effects are also well understood.

Well known is mechanical injury which is the result of destructive forces working during seed handling. Many effects of mechanical injuries are not easily detected because they are confounded with the effects of normal seed deterioration following ageing with time. Direct observations of mechanical injuries will mostly not reveal the full extent of damage.

Injuries would include fractured seed coat, endosperm or mega-gametophyte and embryo parts. Growth tests would show some symptoms as detached seed structures, breaks within struc-tures, abnormally shaped structures, scar tissues, infections, restricted growth, shrinking of cotyledons, and abnormal or broken hypocotyls and radicle. In addition, symptoms would also be reduced viability and irregularities in germination and early seedling development. Again, some types of damage would sometimes be repaired during the imbibition and early germination processes, viz partly broken radicle. Cell membranes tend to stretch extensively or break during rapid drying, and mechanical impact can be specifically destructive to cell membranes under stress from drying.

The nature of mechanical injury varies greatly. The most serious intensive ones reduce viability immediately, whereas the effect of smaller injuries may not be felt at once, but will increase with time.

The extent of damage will differ with species, and with seed moisture content. Thus for corn, where damage was inflicted during processing, a decrease of 3-4% in germination was found for corn of 14% moisture content and a decrease of 70-80% for corn of 8% moisture content! Table 1 shows the effect of cylinder speed of the combine harvester on seed coat breakage of soya bean. Large sized seeds are more prone to damage than small seeds; they are in particular susceptible to injuries that reduce viability. Small seeds are often harvested along with some vegetation which protects them against serious injury. Flat seeds are susceptible to critical mechanical injury. Seeds of a spherical shape are less prone to injury than elongated or irregularly shaped seeds. The number and intensity of impacts during handling would greatly affect the extent of damage.

Effects of mechanical injuries would often be aggravated by a subsequent infection by fungi.

Table 1. Effect of machine speed on seed coat breakage of soya beans.

REVOLUTIONS PER MINUTE	SEED MOISTURE AT HARVEST	
	13.5	12.2
	% SEEDS WITH BROKEN COAT	
700	4	5
900	5	24
1155	12	48

Various types of tests have been developed to expose mechanical injuries. This will be dealt with in the session on seed testing.

The effect of water damage is often confused with the effect of mechanical damage. Water damage refers to damage caused by alternate moistening and drying of mature seeds. The result of water damage is for example cell membrane disruption either due to rapid loss of water of moist living tissue, or rapid uptake of water of dry living tissue. Such damage is most often found in large seeds. The symptoms of water damage could be moist folded seed coats, and the results are loss of germination and seedling abnormalities. Water damage would occasionally be of greater economic importance than mechanical injuries.

There is also some knowledge existing on damage to the genetic systems of seed or the genetic structure of seed populations following seed processing. However, the long term effects of seed processing on the genetic structure of the resulting population of plants derived from the seeds has received little attention. Concern was raised during the preceding IUFRO Symposium in Burkina Faso in 1992, where we heard of genetic selection in a seed population, and where the question was raised as to the effect of all the steps of grading done in seed operations and nurseries.

Seed processing involves a number of operations:

AFTER-RIPENING

EXTRACTION DEPULPING

SCALPING

CLEANING

DRYING

GRADING

INVIGORATING

DRESSING (PELLETING)

It will in the following be discussed how each of these operations may affect seed quality.

Seedlots of trees are often comparatively small - often few kilograms and at most 1-2 tonnes. Agricultural crop seedlots may be of the order of 10 or more tonnes. Tree seed can often be processed rationally by hand and simple equipment. This minimizes the risk for inflicting damage to the morphological and physical structures of the seed, unless of course the process delays other processing of critical influence on seed quality. Sometimes machines may be required for a process, for example where it is impossible to achieve an adequate quality by manual methods, or where there is not sufficient labour available to process the seed within maybe critical short periods. When machines have to be used, the processing often has to rely on machines developed for agricultural crops. These machines will often have a large capacity, and may be irrational for smaller lots, because the essential cleaning of the machine after use may take too much time. Sometimes a large machine cannot even process small seed lots. We see therefore many efforts to develop machinery that should fit the special conditions for small seedlots. The risk of damage to seeds naturally increases when machines are used.

Grading

We shall start with one of the operations that occur rather late in the processing scheme, i.e. seed grading, or sizing as it is also termed. This is because a number of studies have been made on the effect of sizing on seed germination and genetic quality as defined above.

Grading is done using either manual sieving or a variety of ingenious machinery. These work based on seed morphological or physiological seed characteristics as seed dimensions, specific gravity, condition of coat or cell membranes. Well known are the indented cylinder, cylinders with various shaped slots, groves or holes, the gravity separator, draper separator, Dakota seed blower, electronic colour separation, and recently computer aided separation based on very many parameters. Increasingly becoming known are the Pre-vac and the IDS methods (Simak and Bergsten, 1987). Pre-vac separates mechanically damaged seeds from undamaged seeds utilizing differences in rate of water up-take. The IDS method (incubation-drying-separation) separates dead from live seeds utilizing differences in drying rate.

Silen & Osterhaus (1979) working on Douglas Fir, *Pseudotsuga menziesii*, were concerned about the influence of widespread sizing of seedlots on the genetic quality of seedlots. They found large differences in average seed weight between trees (families), but could not separate the effects of the mother tree and the local environment. They found a substantial year-to-year variation in seed size for the individual trees - 15% or more - but still characteristic differences between families. This indicates a strong environmental effect, with an overriding maternal effect. They studied what happened to individual families when seeds were sized. If one-third of the seeds, the lightest part, was discarded then 16 of the 18 families in the study would be affected. Three of these would lose somewhat more than 50 percent of their seeds, and another three lost more than 90 percent. Most importantly, two families among these most affected seedlots were also the top five for 10-year progeny height! The correlation between seed size and 10-year progeny height or diameter was low, being around zero to 0.1. Still, among the one-third families with the heaviest seeds were three families that now ranked high in terms of 10-year progeny height!

Chaisurishi et al. (1992) found for *Picea sitchensis* clones a clonal effect (maternal) on seed size, but heritability was 0,4 only. Among-clone variation accounted for 36 percent of the variation, and within-clones for the rest. Heritability for germinative parameters was 0.7-0.9. Further, they found that seed families differed in their requirements for pretreatment, which indicates clonal differences in seed dormancy. Again, a strong maternal effect on important characteristics.

The possibility of whole families being virtually eradicated in seed sizing operations does not mean that grading should not be done, but perhaps it should be on a family basis. It would be rather impractical and expensive for any seedlot with a good number of families, and this is why total seed quality must include economic weights. The seed customer should be told what it would cost him in regard to loss of genetic variation or in regard to extra operational cost in the nursery, if he choose to grade on a bulk, or on a family basis, respectively.

Sizing of seed bulks is one operation where specific types of seeds are deliberately selected. A number of operations may unintentionally also select certain types of seeds by inflicting damage to the seeds, which may hamper their viability and vigour. Such mechanical injuries are major destructive forces in the reduction of seed quality. Roberts (1972) has revised this aspect.

We will now briefly go through the other operations to see if we can deduce anything about their effect on seed quality.

After-ripening or pre-curing

Whenever the seed collection team leaves a bulk containing many unripe fruits and seeds, proper after-ripening is urgently needed. The immature bulk contains fruits and seed of high moisture content, favouring the growth of fungi, and in extreme cases this may lead to fermentation.

Immature coniferous cones may require long time in the extraction kiln, and even normal kiln temperatures may cause damage to immature seeds. Also the scales of immature cones may fail to react properly to kiln drying thus causing the cones to remain more or less closed and preventing seed extraction. In the absence of proper after-ripening, seed may still remain viable, but incompletely developed seeds will mostly be slowly germinating, and the resulting seedlings may be slow growing; the seeds may be more prone to disease, and tend to produce more abnormal seedlings. Such immature fruits or cones are - after a possible scalping - placed under conditions favourable for maturation. The conditions favourable for maturation are basically the same as those favourable for temporary storage: well ventilated and cool conditions. During pre-curing the crop slowly loses water while the seed still absorbs dry matter from the fruit and completes the ripening process. It is essential not to dry the crop too fast during pre-curing. The surroundings should just be dry and cool enough to inhibit mould growth and active respiration.

During pre-curing, pulpy fruit should be kept in a very thin layer in trays with a non-corrosive wire-mesh bottom or in thin layers in hessian sacks, where they can breathe and where any juice can run off. There should be adequate air circulation and they should at all times be kept as cool as possible (some species are recalcitrant and will not survive cooling below a certain limit). It is recommended to frequently wash off any juice which has developed.

Tectona grandis, Pinus caribaea, Pinus taeda, Pinus elliottii, Pinus merkusiii and *Pinus radiata* are examples of species that benefit from after-ripening. Seed of *Gmelina arborea* may be collected green and can be left until they turn yellow. This will increase the germination percentage and rate.

Since cone and fruit characteristics may be mainly maternally controlled, there may be over-riding family differences for immaturity, and in any case local environmental conditions (light/shade, water) would have strong influence. Families may be lost if proper after-ripening is not done.

Scalping

During or after harvesting, fruits or seeds may be mixed with a variety of material. It is sound practice to remove as many impurities as possible immediately after collection. This initial cleaning is also called "scalping". The cleaning will lower transport and storage costs, will reduce or remove the risk of spreading disease, and facilitate the later processing steps. Many pests and diseases are harboured in branches, twigs, leaves, needles or soil. An example is the dreaded Brown Needle Disease of tropical pines (*Cercoseptoria sp.*) whose gonidiae are found only on the needles, not on the cones or seed. Teak fruits are enveloped in the thin exocarp which adds to the bulk of the seed. Generally, however, tree seed is usually quite clean as compared to many agricultural and horticultural crop seeds.

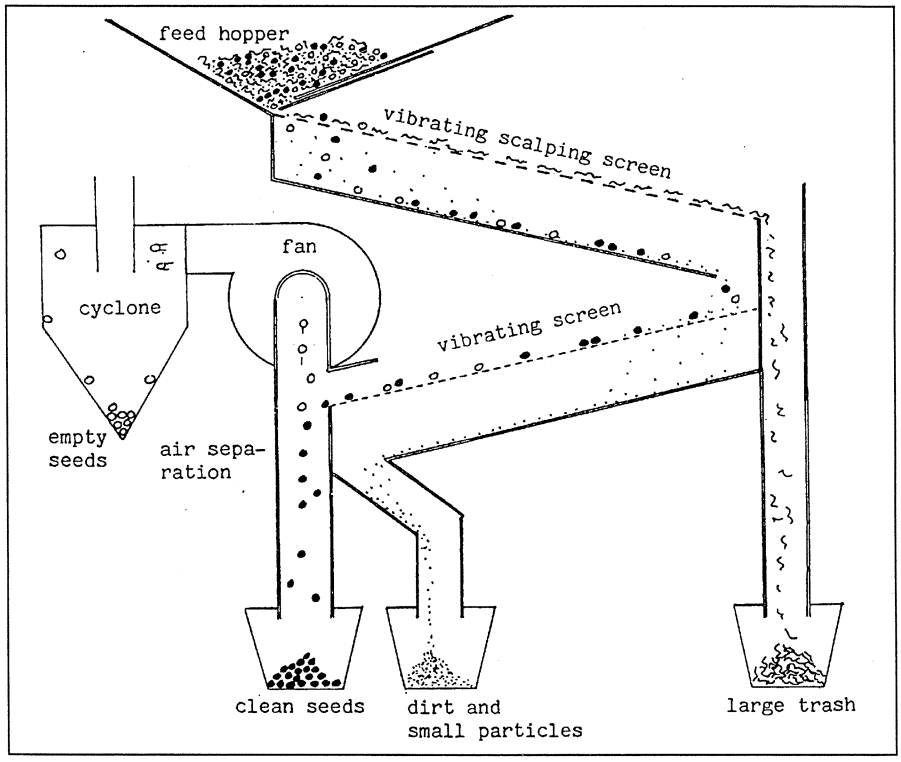


Figure 3 A cleaning or scalping machine

Seedlots from trees may often be comparatively small, and hand cleaning is a simple and effective method. Where larger lots are to be handled, and if there is a shortage of labour and/or time, machines may become relevant. The most simple may be hand operated cylinders with mesh-wire of adequate size. A power operated cleaner, a scalper, is seen in figure 3.

When seeds are handled in machines, there is a risk that thin coated seeds may be damaged. Again, since seed coat thickness may be maternally determined, families could be differently affected. When the operation is done by hand, the cleaning operations would not likely inflict damage to seed of most species.

Extraction

It is rare to collect the seeds themselves directly. Commonly it is the fruits that are collected. Wherever possible, seeds are extracted from fruits and cones, either because the fruit or cone contains many seeds, is too big for handling and sowing, or because the fruit parts may hinder germination, or directly impair viability.

For some species the processing should be delayed so the seeds are kept in the intact fruits as close as possible to sowing in order to retain viability at a high level. This is for example the case of the winged seeds of *Cedrela odorata*.

Fruits and cones can be grouped according to method of extraction. Cones and dry fruits are opened by drying the cone or fruit. Another group consists of pulpy fruits which are macerated to remove the pulp (wet extraction).

Cones

In nature, when seeds are mature, the cone will open when it dries. When the wind shakes the cone, the seed will be dislodged from the cone. If it rains, the cone will close again.

Seeds of cones are extracted simulating the natural system. After possible pre-curing, cones are dried to a moisture level where they start to open, usually at 20-25% moisture content. At 10-15% moisture content the cone scales will normally be fully open. Cones that are collected immature may open at a lower moisture content or not at all.

After opening of the cones the seeds are to be extracted manually or in special machines. Mechanical damage can easily be inflicted on seeds if excessive periods of tumbling or shaking are applied. These activities and the duration of treatment should be adapted to the characteristics of the cone and seed of each species.

Families may differ in their cone moisture content and seed coat thickness and structure, and a standard extraction may affect families differently. We do not know to which extent.

Dry fruits

This is a very diverse group. Examples are follicles that split open down one side, pods from legumes that split down two sides and capsules from eucalyptus that split down three or more sides.

From an extraction point of view dry fruits can be divided into two groups: the truly dehiscent fruit that will split open and expel the seed when dried, and the indehiscent fruit that will remain closed after drying.

There is no sharp line between the two groups. Some species will open readily when dried if they are picked at the right point of maturity; but if they are picked immature or are dried too fast, they will have to be threshed after drying to extract the seed. There are great differences within the groups. Some seeds may be extracted by a light rubbing or tumbling, whereas others may need a series of hard mechanical treatments.

In some cases, the only option may be to extract each seed by hand. If the extraction process becomes too laborious and there are no possibilities of mechanization, one may consider the possibility of storing and sowing the dry fruits as they are.

The removal of the shells is often called threshing. However, in agriculture threshing means the removal of the kernel or pod from the plant, and the removal of the shell is called hulling or shelling. Special machines, de-hullers, have been developed to extract seeds from fruits of difficult structure, see figure 4. The seeds are rubbed against the internal surface of a cylinder. This process may cause much damage to seeds if the machine is not adjusted carefully, but if done properly, a beneficial scarification of seeds may be made.

Again, family differences in seed coat structure could cause differences between families in damage or scarification effect.

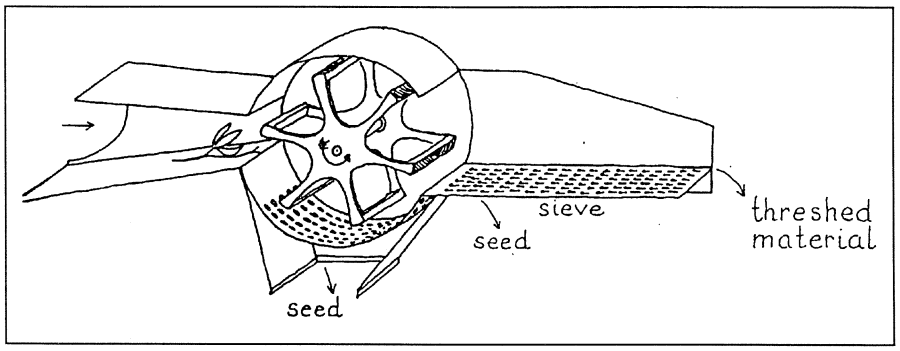


Figure 4 Principle of a dehuller

Fruits with juicy pulp

Species with juicy and pulpy fruits are potentially troublesome. Soon after collection the pulp begins to soften and various fermentation processes may start.

The fruits of many species such as *Azadirachta indica, Cinnamomum camphora*, and *Gmelina arborea* are fleshy and pulpy. It is necessary to have the pulp removed immediately at maturity to prevent loss of germination capacity. Some species such as *Vitex parviflora* may

be dried and stored with the fleshy covering intact, but the germination will normally be improved by depulping.

When mature fruits with juicy flesh are received at the extraction site, they should be processed at once to avoid fermentation and heating. Although fermentation has been advocated sometimes as an aid in depulping and even improving germination (probably by overcoming any seed coat dormancy), it is recommended always to avoid fermentation. Seeds from fruits that have fermented until acetic acid has been formed may be badly damaged.

Nearly all the different methods of depulping require the use of adequate amounts of running water and incorporate maceration, i.e. a more or less gentle abrasion to separate the seeds from the fruit.

From an extraction point of view, fleshy fruits may be divided into two groups: (1) Fleshy fruits with stones, 'drupes', which generally contain one stone with one seed or more. Examples are *Prunus ssp.* (1 seed) and *Melia azedarach* (4 seeds) and (2) fleshy fruits without stones, 'berries', which generally contain many seeds imbedded in the fleshy part without any protection. Examples are berries, pome fruits (e.g. apple) and citrus fruits (e.g. orange). The fruits of the first group are normally easily handled as the stone will protect the seeds. For the second group, the separation is similar to stone fruits, but must be done with much greater care, as the seed is not protected by a stone.

The shape and size of seeds influences the resistance of seed to damage. The smaller and more regular the seed, the less risk of damage. The thickness and structure of the seed coat will of course also influence.

Dewinging

Where dispersal depends on the wind, seeds will often have wings, which usually are removed in order to reduce the bulk during storage and facilitate cleaning and sowing. The method to use will depend on the susceptibility to damage of the seed during the operation and the physical properties of the seed and wing. Mechanical dewinging, if carelessly done, may cause damage to seeds by crushing, cracking or abrasion. Many pine and fir seeds have a small vacuole containing resin. This wall is thin, and may easily be damaged. When this happens, the seed viability may be impaired.

Again, family differences in seed morphology may cause different effects of dewinging.

Drying

When the seeds have been extracted from the fruits, they will still have a high moisture content. Before being passed on to any further processing, orthodox seeds should be dried to an adequate moisture content, whereas recalcitrant seeds cannot tolerate drying, and intermediate seeds may initially be surface dried only. Drying will also facilitate cleaning and upgrading as remains of other matter will not stick to the dry seeds.

Seed drying may be carried out in the shade, in the sun, or artificially in a kiln. Which method to use depends upon the species, the moisture content of the seed, and the climatic conditions (dew point).

When seeds are moist, they are sensitive to high temperatures. The maximum temperature that the seeds will tolerate depends on species and how dry the seed is. Until specific temperatures for a species have been established, a safe maximum drying temperature for most orthodox species is 35°C until the moisture content is below 15%. Below 15% moisture content the temperature can be raised to 45°C and above.

Some typical safe max. temperatures for agricultural species are as follows (Roberts 1972): Cereals like oats and wheat can tolerate 45°C at 30% moisture content progressing to 65°C at 18% moisture content. Seeds of temperate peas and clover will tolerate only 28°C above 20% moisture content and 38°C from 12 to 20% moisture content.

The temperature which the seeds can tolerate also depends on rate of drying since evaporation cools the seed.

In this connection it should be noted that a high temperature also increases the respiration and ageing process. This implies that the period during which the seed is subjected to a high temperature should be kept as short as possible. Where seeds are to be kept stored for extended periods, for example in connection with gene conservation, drying is done for example at a temperature of 15°C and a relative humidity of 15%.

Drying in the shade is to be done when the moisture contents of the seeds is high. There must be adequate ventilation so that the evaporated water can escape. Well ventilated covered walkways or open sheds where rain cannot enter are normally used. To increase the capacity, shelves with trays with a wire mesh bottom may be arranged. The seeds are spread in a thin layer in the trays and stirred regularly.

In certain localities at certain times of the year seeds cannot be adequately dried in the shade, or only with difficulty, because the relative humidity is too high. If the relative humidity is sufficiently low for a few hours during the day, seeds may then be exposed to the air, and if necessary a fan may be arranged. When the relative humidity increases, the seeds must be covered with craft paper or plastic.

Other methods of reducing the relative humidity without raising the temperature are technically more complicated but under some circumstances worth considering. For example for "semi-recalcitrant" seed where the life-span is short already. The idea is either to remove moisture from the drying air (by condensation with a dehumidifier or chemical sorption of the moisture, Silica gel for example) or by lowering the air pressure and thereby increasing the gradient in water vapour pressure between seed and surrounding atmosphere (vacuum drying) if necessary in combination with a temperature control.

In other cases seeds must be dried in an oven, taking precautions to keep temperatures below specific maximum levels as mentioned above.

It is difficult to judge to which extent drying could influence seed genetic quality. However, drying rates could differ depending on (maternally induced) differences in seed coat structure. When seeds are bulked, there would likely be a general stabilization of the moisture content of seeds due to exchange. Where families are kept separate, however, differences in moisture content between families could cause faster aging or deterioration of some families whose moisture content is on the threshold of safe moisture content.

The damage due to rapid drying or moistening of seeds has been described above.

Cleaning

The further processing concerns first of all cleaning for small impurities, which may be fruit remains, and empty seeds.

There are many types of equipment for cleaning, but typically air blowing combined with shaking and sieving are used. Winnowing is a common and age-old manual method. The cleaning processes would normally not inflict damage to seeds.

Seed pre-treatment

Seed-pretreatment to alleviate seed dormancy includes mechanical, physical and chemical agents. Because seed characteristics to be affected by the pre-treatment are very much dependant on the seed mother tree (its genotype and its environment) and differ widely from tree to tree, the effects of pre-treatments would be likely to show a similar wide variation. The result could easily be that whole families may be slower germinating in the nursery than other families, or be non-germinating, resulting in their eradication from the tree populations ultimately established.

Seed dressing, pelleting

Seed of many species are prone to insect or sometimes fungi attacks. Dressing, or treatment, with various forms of insecticides and fungicides is common practice - in fact too common in relation to the actual needs or effects obtained. Application is all too often done without proper knowledge of the biology of the concerned pests or diseases, or of the effects of the chemicals on them, and unnecessary side effects are caused to human beings handling seeds or to the environment.

Damage to seeds are well documented, see for example Jeffs (1986).

On the physiological side, organo-mercury compounds will easily penetrate the seed coat of many types of seeds with a moisture content above 16%, thereby causing loss of viability or distorted seedlings. Seeds with small cracks appear to absorb fungicides more easily than sound seeds. The effect increases with time of exposure. Sometimes chemicals may induce dormancy, and a lowered apparent viability may be confounded with dormancy. Methyl-bromide is well known for its harmful effect to seeds. The harm may be due to an overdosis, or a prolonged exposure due to lack of proper aeration after treatment, or it may be due to too high a moisture content in the seeds. Fortunately, the use of many of the most harmful compounds are to an increasing extent being prohibited or dis-recommended because of their negative effect on the environment.

Obviously, where families differ in their seed coat structure and intactness, treatment with chemicals may cause different effects on families in seed quality. The differences may even be exaggerated because healthy seeds may benefit from the treatments, while the viability and vigour of the unhealthy seeds may be impaired.

Some sticker compounds, as used for example in pelleting operations, have been shown to promote the absorbtion of chemicals into the seeds.

6. SEED CUSTOMER AND SEED QUALITY

A seed customer wants to make a plantation with trees, which survive, produce and look like the trees in a specific stand (populations of trees) he has seen. It is general knowledge that if seed collection is properly done, i.e. if a good proportion of genes in the seed mother tree population is obtained, the progeny would show quite similar performance and characteristics as the mother trees in similar environments.

In practice, however well a seed collection is performed, it will not be possible to obtain a 100% representation of the genes of the mother tree population, and the frequency distribution of genes of the seedlot would most likely be different from the mother tree population. However, a high proportion of genes, as much as 90% of the existing genes, could be obtained in a well performed seed collection. This may be sufficient to ensure that the major characteristics in respect of adaptation, growth and appearance of the mother tree population is transferred to the new plantation.

The seed customer may unknowingly partly be counteracting his efforts to secure a specific genetic variation by putting forward a number of other requirements on the seed lot. For example, he may not tolerate lower than 80% germination in his nursery, or he may not be able to provide optimal conditions for germination. This would require the seeds to be cleaned of low vigour seeds. He may wish to sow by machine in open beds, and for this require seeds of a certain minimum and fairly uniform size. This would need seeds to be graded. Any of these operations may change the genetic composition of the resulting seed lot, hence the plants obtained.

Evidently, already when seeds are received from the collectors, the genetic structure is different from that of the seed mother tree population. Whatever the situation is, we would like to keep the achieved genetic variation at the level it is when seeds are received. However, for species where much processing is done, we may cause unintended changes in the genetic quality using traditional processing methods in our effort to improve physical and physiological quality. The problem is that we do not know the kind and extent of changes we may make by seed handling operations.

It is not surely known what the implication of changes in the genetic structure of tree populations would be on the performance of the resulting plants. Implications would be different whether we are concerned with long or with short term aspects. It would also be different for natural populations of wide genetic variation than for trees which have been intensively improved.

In addition to unintended changes to the genetic structure of a seedlot, processing operations may cause damage to the physiological and morphological systems of seeds. This is possibly the most widely and best studied aspect of seed quality.

Seed suppliers and seed dealers are essentially responsible for the quality of the seed supplied, and ought to provide the seed customer with information of the possible consequences his particular requirements may have on the quality of the seeds supplied. We need to know about these consequences before we can provide good advice to the seed customer, whether private, company or society!

7. REFERENCES

Bonner, F.T., 1977. Equipment and supplies for collecting, processing, storing, and testing forest tree seed. Southern For. Expt. Gen. Tech. Rep. SO-13, Stat., U.S. Dept. Agric., For. Service.

Chaisurishri, K., Edwards, D.G.W. and El-Kassaby, Y.A., 1992. Genetic control of seed size and germination in Sitka Spruce. Silv. Gen. 41/6.

Edwards, D.G.W., 1982. Impact of seed quality on costs of nursery stock. Canadian Forest Service, Pacific Forest Research Centre, Victoria, B.C., Canada.

El-Kassaby, Y.A., Edwards, D.G.W. and Taylor, D.W. 1992. Genetic control of germination parameters in Douglas-Fir and its importance for domestication. Silv. Genet. 41/1.

Fenner, M. (edt.), 1992. Seeds - The ecology of regeneration in plant communities. C.A.B. International, Oxford, England.

Fystro, I. 1979. Klengning og frøframstilling. Lecture note, Statens Skogfrøverk, Hamar, Norway.

Huber, R.F. 1981. High-quality collection and production of conifer seed. Inf. Rep. NOR-X-235. *In*: Proc. Workshop, Edmonton, Alberta, Canada.

ISTA, 1975. Survey of equipment and supplies. ISTA Secret. Zürich, Schweiz.

Jeffs, K.A., 1986. Seed Treatment. BCPC Publications, Surrey, U.K.

Lowman, B.J., 1975. Equipment for processing small seedlots. USDA Forest Service, Equipm. Dev. Centre, Missoula, Montana, U.S.A.

Prasad, R. and Kandya, A.K. 1992. Handling of forest seeds in India. State For. Res. Institute. Assoc. Publ. Company, New Delhi, India.

Roberts, E.H. 1972. Viability of seeds. Chapman & Hall, London.

Silen, R. and Osterhaus, C. 1979. Reduction of genetic base by sizing of bulked Douglas-Fir seed lots. Tree Planters Note 1979/24.

Simak, M. and Bergsten, U. 1987. Sortering och vitalitetshöjning av frö. Report no. 17, Inst. f. Skogsskötsel, Agric. Univ., Umeå, Sweden.

Stubsgaard, Finn 1989. Seed Handling Prior to Processing, Lecture Note C-6, Danida Forest Seed Centre.

Stubsgaard, Finn and Poulsen, Karen M. 1995. Seed Moisture and Drying Principles. Lecture Note C-5, Danida Forest Seed Centre.

Stubsgaard Finn and Moestrup, S. 1991. Seed Processing, Lecture Note C-7, Danida Forest Seed Centre.

Thomson, J.R. 1986. An introduction to seed technology. Edinburgh School of Agriculture. Leonard Hill. Publ. U.K.

Wang, Xiao-Ru, 1992. Genetic diversity and evolution of Eurasian *Pinus* species. Dissertation, Swedish Univ. of Agric. Scien., Faculty of For., Dept. of Genetics & Plant Physiol., Umeå, Sweden.

Willan, R.L. 1985. A guide to forest seed handling - with special reference to the tropics. FAO Forestry Paper 20/2, Rome.