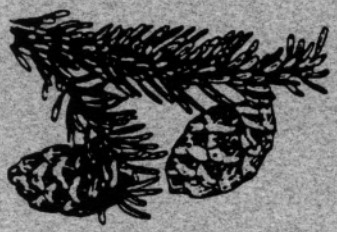
CANADIAN TREE IMPROVEMENT ASSOCIATION/
ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES



Tree Seed Working Group

NEWS BULLETIN

No. 30, May 1999

A WORD FROM THE CHAIRMAN

Our maritime climate in Nova Scotia has always proved to be very difficult to work with; this year is no exception. The weather used to be: 'wait 15 minutes and it will change'. We recently experienced a week of daytime highs reaching 27 degrees C, while this week I was placing pollination bags on black spruce trees as it snowed. So, I can't wait until next week to see what the weather will throw our way. This spring, there were reports of wells already dry in the western end of the province. We are setting up an irrigation system for a black spruce orchard located in the Annapolis Valley, a job we usually don't consider this until the end of June. Enough news about the weather out east - time for seed matters.

Lately, there has been a great deal of interest in the propagation of black ash. We participated in a small seed collection last fall and have had some success, producing about 150 seedlings. We will continue working with this species as well as white ash in an attempt to produce these species on a regular basis. Our goal is to achieve consistent, uniform seed germination.

Demand for improved seed continues to increase as the number of trees being planted in Nova Scotia has risen again. Rabbits or snowshoe hare have been having a devastating effect on some of our plantations, especially those of Norway spruce. Just to keep things interesting, the new gas pipeline, which is crossing the province, managed to go right through the middle of one of our oldest and best black spruce family tests. Never a dull moment in the tree breeding business!

Nineteen ninety-nine represents the first time in a couple of decades that the CTIA/ACAA will 'miss' holding its biennial meeting. However, not all is lsot as we have one scheduled for the year 2000 in Sault Ste. Marie, Ont.(see later in this issue for more details). In the interim, if you have any ideas or suggestions, please send them along to Dennis Joyce.

Enjoy your summer.

Howard Frame

EDITORS NOTES

I am pleased to start off my few words of wisdom by NOT lamenting about how difficult it has been to solicit contributions for this issue of the NewsBulletin!! Between comments I received regarding my last diatribe on **"Tree Seed Research: Is anyone doing any?** " and responses to a note on the TREESEED DISCUSSION GROUP, I received 'plenty' of information, articles, etc. for this issue. THANKS TO ALL THOSE WHO MADE CONTRIBUTIONS.

This spring has been one of the earliest and warmest on record for those of us from "Out East". If this hot and dry weather holds for a couple of more months (wishful thinking?), 2000 should be a very good year for cone production.

We should all be pleased to know that Dennis Joyce, Randy Ford and their cast of thousands are active in planning our millenium CTIA/ACAA

meeting (see Upcoming Meetings for further details). I am sure that the meeting will be interesting and educational and that we will be treated to great hospitality. Be sure to jot the dates down now.

What about a Tree Seed Working Group Workshop in 2000?

We need to start looking now, for a theme for a workshop IF we are going to be able to have one. Do any of our readers have any suggestions? Although a theme for the CTIA/ACAA meeting has not yet been finalized, Dennis did mention that breeding and tree improvement strategies will be emphasized. Perhaps the focus of our workshop could be the various strategies for the continued use of seed as part of a delivery system for reforestation planting stock?

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Failed Seeds make perfect baby food

(The following tidbit was taken from New Scientist Dec. 12, 1998).

The huge crop of seeds produced every few years by Norway spruce help tree seedlings to gain a foothold by fertilizing the forest floor, say researchers in Sweden.

Most of the seeds germinate and die immediately, releasing nitrogen into the soil. Olle Zackrisson and his colleagues at eh Swedish University of Agricultural Sciences in Umeå simulated a heavy seed crop by adding killed Norway spruce seeds to plots in the boreal forest of Sweden. With added seeds, and where the forest floor was free of moss, Scots pine seedlings grew more than 50 percent larger and had a higher nitrogen content compared with seedlings on control plots. In mossy lots, only the moss benefitted from the extra nitrogen (Oikos, 84:p.17)

[Thanks to Peter Hall for sending this article]

TREE SEED WORKING GROUP

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Comments, suggestions and contributions for the Newsletter are welcomed by the Chairman or editor.

The Association of Official Seed Certifying Agencies (AOSA)

The website for AOSA is:

www.aac.msstate.edu/mafes/aosca/

Seed Handling Guidebook

The BC Ministry of Forests, Tree Improvement Branch has initiated the production of a **Seed Handling Guidebook**. This guidebook is intended for those who directly handle cones or seed (collectors, orchardists, processors and nurseries) and to those who would benefit from an integrated view of seed handling from cone collection to nursery sowing of seed (owners, reforestation foresters).

This guidebook will address three main issues:

- seed handling guidelines
- tools and resources required to recognize seed problems, and
- techniques used to avoid or correct seed problems

These issues will be investigated in the context of a seed handling system that covers all aspects of seed handling from cone collection to sowing in the nursery (Figure 1). Most of us deal with only part of this system, but it is important to understand the full spectrum of seed handling activities as poor handling at any stage may impact your product!

The following sections will be written by the indicated people:

Seed Condition; Seed Processing; Seed Storage; Seed Pretreatment - **Dave Kolotelo**

Seed fungi; Collections and Post-Collection Handling - **Mike Peterson**

Other Seed Treatments; Seed Sowing - **Dave Trotter**

Seed Insects - **Robb Bennett**

John Dennis is also actively involved in this project by providing operational data and his expertise with regards to seed pathology.

We would like this guidebook to represent the **best available knowledge** on seed handling. If anyone has **operational information** that would be helpful to individuals involved in seed handling we would like to include these in the Seed Handling Guidebook.

Please forward any tips, tricks or suggestions to myself, or any of the other authors, for inclusion in the guidebook. This call is intended for those techniques that do not make it into the scientific literature, but still play a valuable role in seed handling.

Thank you for your input!

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Call for Nominations for the IUFRO Seed Physiology and Technology Research Group

The following two tidbits were taken from the IUFRO Seed physiology and Technology Newsletter No. 52.

Elections

Chairman George Edwards raised, as the main topic for this issue, the need to establish a new Executive for the Research Group (IUFRO Research Group 2.09.00). George notes that the present Executive (he as chair and two co-chairs) have served for 10 years and the 'Statute of Limitations' so-to-speak has run out.

The deadline for nominations for the term 2001-2005 is October 31, 1999.

State-of-the-Knowledge Report

IUFRO President, Jeff Burley, has issued a challenge to all IUFRO Divisions, Research Groups and other units to prepare a "State-of-the-Knowledge" report in time for the World Congress in 2000.

All readers who are affiliated with IUFRO and seed research should take a moment to prepare a brief report and submit it to George Edwards. Further details are given in the Newsletter.

For more information on the research group, please contact the Chairman

Dr. D.G.W. Edwards
FTB Forest Tree Beginnings
4018 Cavallin Court
Victoria, B.C.
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Upcoming Meetings

Seed Biology Workshop

The Ohio State University Seed Biology and Extension programs will host a Seed Biology Workshop July 8 and 9, 1999, on the Columbus,Ohio campus. The workshop will feature "hands-on" sessions covering germination and vigor testing, tetrazolium tests, seed enhancements, and plant health.

We will also feature presentations on the technological aspects and challenges of optimizing the quality of high value seeds and new developments in seed imaging research. These presentations will include time for audience participation. We anticipate lively discussion sessions that will provide an avenue for the exchange of information and ideas.

The workshop will be credited towards RST training/continuing education by the Society of Commercial Seed Technologists. Two hours of credit can be earned per day for a total of four units. For further information contact:

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Fax. (614) 292-7262

or

Andy Evans
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Thanks,

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TheThird International Workshop on Desiccation-sensitivity and -Tolerance In Seeds And Vegetative Plant Tissues

January 3rd to 15th , 2000
South Africa.

To facilitate reservation of a venue and costing of the meeting, we need to know - as soon as possible - about your intention to participate. For those who have not yet responded to the first announcement, we'd appreciate your indication of interest to the following email address:

deswork@biology.und.ac.za

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Correspondence Courses for Seed Analysts

Steven Fennimore: The Division of Educational Outreach at Colorado State University offers 4 correspondence courses for beginning seed analysts. These courses cover seed technology and seed development and metabolism. The title of the courses are:

1) Seed Anatomy and Identification
2) Seed Development and Metabolism,
3) Purity Analysis, and
4) Germination and Viability.

Information on these courses can be obtained on the internet at:

www.colostate.edu/Depts/CE

Seed Workshops

Colorado State University, Division of Educational Outreach has two workshops scheduled for July. The Seed Identification and Purity workshop is July 12 - 16 and the Seed Viability workshop is July 19 - 23, 1999 at Fort Collins CO. Contact the Division of Education Outreach for more information 1-800-525-4950.

In addition to these two workshops, a two day educational tour will be available July 17 and 18, 1999. The tour will take students to two locations within the Colorado Rocky Mountains to see the revegetation work being done at the Colowyo mine and to tour the Upper Colorado Environmental Plant Center. At both of the site students will be able to identify plant species and collect seeds.

Loren Wiesner
National Seed Storage Laboratory
Fort Collins, Colorado, USA

The interaction Between Nursery Management and Silviculture Operations

IUFRO Subject Group 3.02.00 "Operational methods in the establishment and treatment of stands"

September 28-30, 1999
Auburn University Hotel and Conference centre
Auburn, Alabama

For more information please contact

Ken McNabb, Co-Cordinator
IUFRO Subject Group 3.03.00
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Tel: (334) 844-1044
Fax: (334) 844-1084
Email: mcnabb@forestry.auburn.edu

28 - 30 September 1999

Western Forest Genetics Meeting

WFGA 1999 Annual Meeting
July 17 to July 19, 1999
Southwest Forest Science Complex
Northern Arizona University
Flagstaff, Arizona

Registration forms and information about the WFGA meeting can be found on the web site located at

http://www.for.nau.edu/forestry/WFGA_Conf99.

For more information please contact

Dr. Laura DeWald
Tel:(520)523-8129
Email: Laura.DeWald@nau.edu

Canadian Tree Improvement Association Meeting

August 14-17, 2000,
Sault Ste. Marie, Ontario

Theme: Genetic Resource Management: Focusing on Strategies.

For more information please contact

Dennis Joyce
Ontario Forest Research Institute
1235 Queen St. E.,
Sault Ste. Marie, Ont.
P6A 5N5
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The World Seed Conference

September 6-8, 1999
Cambridge, England

For more information please contact

Secretariat
42 Devonshire Road
Cambridge, England
CB1 2BL
Great Britain, United Kingdom

Tel: +44/1 223 323 437
Fax: +44/1 223 460 396
Email: cc@confcon.demon.co.uk

or

visit the web site at
http://www.worldseed.org/~assinel/wsce.htm

Second Symposium of Advances on Forest Seed Production in Latin America [II Simposio avances en la producción de semillas forestales en América Latina]

October 18-22, 1999
Santo Domingo, República Dominicana

For more information please contact

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Third International Workshop on Dessication-Sensitivity-Tolerance in Seeds and Vegetative Plant Tissues

January 2000
University of Natal, South Africa

For more information please contact the organizers:

Fax: +27 31 260 1195
Email: deswork@biology.und.ac.za

Storage of scarified seeds of *Leguminosae* trees and shrubs

Introduction

A large number of tree seed species do not germinate due to hard seed-coats (physical dormancy) hindering uptake of water (Bonner *et al.* 1974, Poulsen and Stubsgaard 1995) and the seed will not germinate unless the seed-coat is scarified. However, unaware selection is often performed when scarifying *Leguminosae* seeds through widely used pre-sowing treatments (*e.g.* soaking in boiling water or sulphuric acid). Due to high variability in hardseededness, such treatments often tend to damage only those seeds exhibiting delicate seed-coats and can thus turn into genetic erosion. Furthermore, some of these procedures are too risky and not suitable for unskilled workers ; health hazards involved in working with sulphuric acid or with large amounts of boiling water are special problems to be considered (Poulsen and Stubsgaard 1995).

Materials and methods

To avoid any selection, an electrical mechanical scarifier (the Forsberg scarifier), modified *ad hoc* to provoke even and shallow cracks on the seed-coats, was employed to scarify seeds of four legume species: *Acącia cyanophylla*, *Ceratonia siliqua* (carob), *Laburnum anagyroides* (golden chain laburnum) and *Robinia pseudoacacia* (black locust). Before beginning the experiments, small-scale trials were conducted to identify the most appropriate length of scarification (5, 15, 30, 45 or 60 seconds) and sandpaper type (Fiar Corindone no. 40, 60, 80) for each species. The speed of the internal blades was always set at 1,425 rpm.

Untreated or mechanically scarified seeds were stored for 0, 6, 12 or 18 months at +3°C or -3°C, in sealed containers or by vacuum packing. Thirty-two different treatments, studied through a multifactor design, were experienced for each species. Factor A was seed scarification with 2 levels: mechanically scarified seeds and non-scarified seeds. Factor B, with 2 levels, was storage container type: hermetically sealed container and vacuum packing. Factor C, with 2 levels, was storage temperature: +3°C and -3°C. Factor D, storage length, had 4 levels: 0, 6, 12 and 18 months.

Following the seed scarification, and after 6, 12 and 18 months' storage, germination tests using 6 replications of 100 seeds each were carried out. The same procedure was applied for non-scarified seeds. The germination tests were conducted as prescribed by ISTA (1996) rules.

Germination Percentage (G%) was calculated, Djavanshir and Pourbeik's (1976) Germination Value (GV) being measured as well. GV combines speed and completeness of germination into a single index: the higher the GV, the more rapid and complete the germination.

A germination test employing manually chipped seed, as prescribed by ISTA (1996) rules, was carried out for each species; 6 replications of 100 seeds each were used.

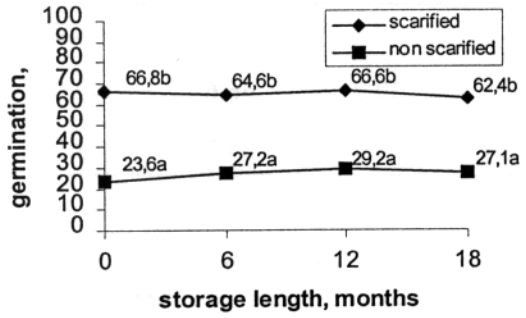
Results and discussion

Ceratonia siliqua (carob)

As no loss in germination was observed (Figure 1), it seems reasonable to assume that storage for at least 18 months is highly safe for scarified seeds viability. Considering the effect of single factors on germination, no influence was recorded for storage temperature and duration. While mechanically scarified seeds behaved much better than non scarified ones, seeds stored in hermetically sealed containers germinated more than those vacuum packed (Piotto & Piccini 1996).

It is useful to examine factor A (scarification) x factor D (storage length) interaction, that is the influence of duration of storage on scarified and non scarified seeds (Figure 1). G%s and GVs were far better for scarified seeds with respect to non scarified ones, but within each of these two groups no differences were observed with relation to storage length (0, 6, 12, 18 months). For instance, germination of scarified seeds stored for 0 months (66.8%) was not significantly different from those stored for 18 months (62.4%). Seed damage due to the modified Forsbeg scarifier seems to be negligible as germinative capacity of mechanically scarified seeds (65.1%) is similar to that of seeds subjected to germination test as prescribed by ISTA (1996) rules (67.5%).

A.



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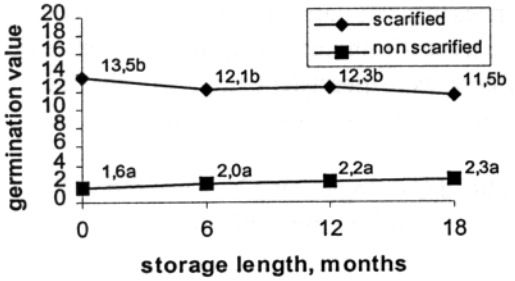

Figure 1. Effect of scarification (A) and storage length (B) on germination percentage an Djavanshir and Pourbeik's germination value of *Ceratonia siliqua* L. scarified and non scarified seeds. According to the Tukey's test, means followed by the same letter within each line, are not significantly different at $P<0.01$.

Laburnum anagyroides (golden chain laburnum)

Storage up to 18 months in hermetically sealed containers seems highly safe for scarified seeds viability. Results are going to be presented in detail in September at the 1999 World Seed Conference (Cambridge UK).

Acacia cyanophylla and ***Robinia pseudoacacia*** (black locust).

Processed unpublished data suggest that the behaviour of scarified seeds of *Acacia cyanophylla* and *Robinia pseudoacacia* after a 18 months' storage resembles the trend described for carob and golden chain laburnum.

Conclusion

This technique, probably suitable for other *Leguminosae* seeds, expresses a good utilisation of simple seed technology and gives nurserymen a useful tool for better scheduling sowings avoiding losses of genetic variability. Furthermore, the treatment can be performed quite quickly, if compared to other mechanical scarifying methods, and temperature requirements to storage safely scarified seeds can be fulfilled by normal refrigerators. This could be of interest in developing countries.

References

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Piotto, B. and Piccini, C. (1996). Storage of scarified carob seeds : influence of container, temperature, and duration on seed quality. *Fruits* 51, 261-267.

Poulsen, K.M. and Stubsgaard, F. (1995). Three methods for mechanical scarification of hardcoated seed. Humlebaek, Denmark, Danida Forest Seed Centre, Technical note 27, 15p.

Willan, R.L. (1990). Seed pretreatment. Humlebaek, Denmark, Danida Forest Seed Centre, Lecture note C-10, 19 p.

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National Tree Seed Centre

Centre staff were busy over the winter months processing seed collected last year and conducting germination tests. Testing was done on newly collected seed lots but the bulk of the work was focused on seed lots which had not been tested for 8 to 12 years. Significant progress is being made but it will be several more years before the backlog is completed.

An undergraduate student completed her thesis which was a storage experiment of red oak acorns. Oak is considered to be a recalcitrant species when it comes to storage. This means that acorns cannot be dried; rather their moisture content must be maintained in order to maximize viability. Storage for more than 6 months is not usually a concern for nurseries but is necessary for seed banks.

Acorns were collected from three trees at one week intervals for three weeks. Acorns were stored in glass jars and invigoration tubes with and without peat. The glass jars were 1 litre Mason jars while the invigoration tubes were pieces of 10 cm diameter PVC pipe 30 cm long. Gortex was used to cover both ends of the invigoration tubes and the mouth of the jars. Enough acorns were stored to permit sampling every 6 months for 24 months. She reported results after 6 and 12 months.

Moisture content and germination of the acorns declined over time with the greatest decrease for the acorns stored in the invigoration tubes. The two tables below show results for acorns stored in glass jars.

Table 1. Germination (Germ) and moisture content (MC) (%) of acorns stored in glass jars without peat after 0, 6 and 12 months

	0 mos.	6 mos.	12 mos.
Germ		87	54.8
MC	39.2	39.3	34.6

Table 2. Germination (Germ) and moisture content (MC) (%) of acorns stored in glass jars with peat after 0, 6 and 12 months

	0 mos.	6 mos.	12 mos.
Germ		100	48.4
MC	39.2	46.6	42.3
MC peat	73.4	34.7	38.5

Moisture content of acorns stored with peat was higher probably because they absorbed moisture from the peat as seen by the decline in moisture content of the peat. Germination showed a decline from 6 to 12 months for both storage treatments.

Differences in germination of acorns collected from the three trees diminished with time but collection date effects were persistent implying that acorns must be mature before collection commences.

The Seed Catalogue may be accessed at the following web site:

http://ultratext.hil.unb.ca/Texts/Forest/MX203/english/title.htm

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IUFRO Seed Physiology and Technology Research Group Home Page

http://iufro.boku.ac.at/iufro/iufronet/d2/hp20900.htm

Feature Article

Testing of tree seeds for germination

I read Dave Kolotelo's article on " Stratification in germination dishes" (Tree Seed Working Group News Bulletin No.29, November 1998) with interest and decided to write this short note to offer my many years experience in testing tree seeds for germination at the former National Tree Seed Centre, Petawawa National Forestry Institute. Since seed testing is still an important part of our important seedling production for regeneration, I thought some of my experiences may be useful to those who are doing seed testing.

The objective of testing seeds is to evaluate the quality of the seedlot in terms of viability or germinability in order to calculate the sowing rate. Seed is a living biological end-product of genetic and environmental interaction, and its behaviour cannot be predicted with certainty. Therefore, the methods used for testing must be based on a scientific approach, accumulated knowledge and experience of the seed analyst, accuracy and reproducibility. Under these principles, seed testing has to be carried out efficiently and economically.

Viability tests are rapid testing of seeds for the percentage of viable seeds in a seedlot. They are indirect tests such as tetrazolium staining (TZ), excised embryo, hydrogen peroxide, x-ray and cutting. Seed viability should be distinguished from germinability as viable seeds are not necessary germinable. The viability tests are designed for quick testing of seed viability of dormant seeds or there is time limitation. For viability testing, readers can consult the international rules for seed testing (ISTA 1996), handbook of tree and shrub seed testing (Gordon et al. 1991), handbook on tetrazolium testing (Moore 1985), quick tests for tree seed viability (Leadem 1984), rapid viability testing of tropical tree seed (Bhodthipuks et al. 1996) and tetrazolium testing of agricultural seeds (Grabe 1970).

Germination tests are the direct growth tests to determine the maximum germination potential of a seedlot under prescribed controlled laboratory conditions. For dormant seeds, proper pretreatments are required to overcome seed dormancy before testing. Because tree seeds of many northern temperate regions are dormant in various degrees, they require a period of moist chilling (cold stratification) to release their dormancy for maximum germination. For routine laboratory testing for germination, most ISTA accredited laboratories follow the following procedures:

1. Reduce the test bulk seeds to about 400-800 seed sample (depending on seed dormancy) with a seed sampler (Boerner sampler, soil divider) or by hand;

2. Draw four replications of 100 seeds each from the reduced seed sample using the vacuum counting head and directly place the seeds onto the moist germination medium (blotters, filter papers or Kimpak) in a germination box. After all four replications of 100 seeds each are properly placed in the germination boxes, they are placed in a cold room (2-5^{0}C) for moist chilling in the dark or in germinators at prescribed temperatures, relative humidity and light. Dormant seeds requiring various periods of moist chilling pretreatment, are moved to the germinators following their required chilling period.

3. During the germination test period, germination progress is monitored periodically. ISTA rules suggest first germination counts at 7 days and final counts at 14, 21, 28 or 35 days depending on the test species.

4. At the end of the test period, ungerminated seeds are cut to determine the number of fresh, empty, insect damaged or dead seeds; or using a tetrazolium test to determine viable seeds.

5. Calculate and report test results of the germination test by calculating the percentage by number of normally and abnormally germinated seedlings, and ungerminated fresh, empty and dead seeds. Before the test results are reported, the average and range of the germination data of the four replications are calculated to check the reliability of the test.

The difference in germination between the lowest and the highest replicates is compared with the established ISTA Tolerance Table to see if it is within the accepted tolerance. A retest is required if the difference of lowest and highest germination replicates exceeds the established tolerance. The difference between the two tests is compared with the established tolerance level for two germination tests of the same seed sample to check their compatibility.

The above are normal routine procedures followed by most seed testing laboratories but there are variations among laboratories concerning germination medium, moisture level, germination box, germination cabinet with various degrees in temperature fluctuation, sowing method, germination criteria and even in interpreting test results. I would like to discuss these issues briefly.

Germination medium

ISTA rules prescribe paper substrates, sand and soil with general specifications for composition, texture, strength, pH, storage and sterilization; and quality control. For convenience of handling, high moisture holding capacity and ease of monitoring, many of the seed testing facilities in North America prefer using Kimpak (Cellulose wadding paper) as a germination medium. I find this medium more suitable for testing coniferous and small seeded hardwood seeds better than others as it allows seed analysts to assess root abnormality and ease of evaluation. In developing countries many seed testing and research laboratories use sand or paper towels for germination medium as filter papers and blotters are an expensive imported material.

Germination medium moisture

ISTA rules specify sufficient moisture to meet the requirements for germination. The optimum initial quantity of water to moisten the germination medium should be determined by experiment. Subsequently watering should be avoided wherever possible as it is likely to increase variation among replicates. Therefore, precautions must be taken to ensure that the medium should not be allowed to dry out and sufficient water is supplied continuously during the test period. Excessive water should be avoided or aeration may be restricted.

Over the years I have found that the initial quantity of water should be standardized with a fixed amount that will last throughout the period of moist chilling and the test period without having to add additional water to the germination medium. At the former National Tree Seed Centre, we standardized the distilled water quantity for each Kimpak germination medium in each Petawawa germination box at 110 mL with another 110 mL in the water reservoir. The fixed amount of water can be efficiently dispensed evenly over the germination medium with a peristaltic pump thus all four replicates receive the same amount of water. This amount of moisture has been found appropriate for practically all coniferous and small hardwood seeds. We never had to add any water to the germination medium throughout the moist chilling and germination test periods. We believed this helped in improving the rate and uniformity of germination and minimizing the variability among replicates. However, the germination box has to be covered properly.

Germination box or tray

There is no specific suggestion on germination containers and there is no containers made commercially for seed testing available on the market. Therefore, seed testing laboratories are using all kinds of containers locally available from petri-dishes to various kinds of plastic or metal containers. At Petawawa, we searched and tested several types of containers for tree seed germination and failed to find even one suitable for research or routine germination tests. After two years of research and development with the help of the PILP program, we developed the Petawawa germination box which was designed for seed germination tests (Wang and Ackerman 1983) and has been widely used in different countries worldwide. The uniqueness of this germination box is its dimension, durability and light weight, water reservoir and universal locking system to allow for full seedling growth according to ISTA normally germinated seed criterion. Many of the commercially available plastic containers such as petridishes are too small and shallow for germinating seeds to develop to full seedling stage. It is especially true for seeds prone to fungal development (e.g. true firs, Douglas-fir, eastern white pine, white spruce). Furthermore, the lid and the bottom of the Petawawa germination box is fit to permit air exchange.

Pretreatment to break dormancy

There are more types of dormancy known in agricultural crop seeds than in forest tree seeds due to much longer breeding and advanced research in seed physiology and biochemistry of the former. Therefore, the treatment prescriptions for breaking seed dormancy in the ISTA rules include dry storage, prechilling, preheating, light, potassium nitrate, gibberelic acid and sealed polyethylene envelopes for overcoming physiological dormancy; and soaking, mechanical and acid scarification for hardseedness; and prewashing and removal of structures around the seed for removing inhibitory substances. Although some of these treatments are used for releasing tree seed dormancy such as mechanical or acid scarification for removing seedcoat dormancy in honey locust and black locust or a combination of removal of fruit coat, soaking in acid and GA_3 and prechilling for breaking a combined physical and physiological dormancy in basswood seeds (Pitel and Wang 1988); prechilling is the most frequently used method for breaking dormancy of most coniferous and small hardwood seeds. Place sown seeds for germination tests at 1-5°C for a period ranging from 2 weeks to 12 months depending upon species; although ISTA does not recommend pretreatment of more than 2 months for germination tests. It recommends that deep dormant seeds be tested by alternative viability methods such as tetrazolium staining, excised embryo or x-ray tests.

For proper moist chilling (cold stratification) treatment of tree seeds to overcome dormancy, it is critical to allow seeds imbibing sufficient moisture and to provide a moisture supply continuously during the prechilling period, especially for relatively longer prechilling periods. The continuous supply of moisture during the moist chilling period is important for seeds of deep dormancy (Nikolaeva 1969). For example, it took the excised embryo of sugar pine (*Pinus lambertiana)* 30 days to imbibe sufficient moisture from a moist vermiculite in the first phase of water uptake, followed by 30 days of no water uptake in the second phase 30 days and gradual increase in water uptake in the third phase of 30 days when the moisture content of the embryo reached 57% of dry weight (about 36% fresh weight) (Stanley1958). For white spruce seeds, the established critical level for effective prechilling is between 20% and 25% moisture content (fresh weight) (Downie et al., 1998). The currently practised naked cold stratification in plastic bag technique which involves presoaking seeds for 24-48 hours prior to prechilling requires more handling (soaking, draining, transferring, aerating and checking seeds throughout the prechilling period). The recently published modified prechilling methods with target moisture (Bergsten 1987; Downie and Bergsten 1991; Jones and Gosling 1994) provides a gradual water imbibition and avoiding possibly soaking injury (i.e. true firs) (Jones et al. 1991; Gosling 1997). Jones et al. (1991) consider the causes of soaking injury to *Abies procera* seeds could be a number of facors including the temperature of soaking, rate of water uptake, oxygen availability during soaking, acting either singly or in combination. In an earlier extensive review of temperature and seed dormancy, Stokes (1965) reported that the detrimental effect of excessive water absortion on germination is very likely to be due to reduced eccess of oxygen, particularly when the presoaking is taking place at high temperatures.

For moist chilling temperature regime, ISTA prescriptions recommend 1-5°C or 3-5°C for seeds of all tree and shrub species (Part 2, Table 5, ISTA 1996). However, we must keep in mind that for seeds requiring longer moist chilling period, they would be better chilled at lower temperatures (1-2°C) to avoid germination during chilling period (Allen 1960; MacArthur and Fraser 1963; Danielson and Tanaka 1978).

Method of sowing

In most seed testing laboratories seeds are frequently sown by using counting boards or vacuum counters. The former is more commonly used for agricultural crop seeds than for forest tree seeds. The vacuum counter is very commonly used in seed testing laboratories because it is efficient, unbiased, accurate, and allowing sown seeds in even space and close contact with the moist germination medium thus facilitating the germination process and ease of evaluation of germination progress. There was some fear about

the use of vacuum counters because they may be biased by picking empty or underdeveloped seeds. However, with today's advanced seed processing technology and efficient seed handling, we can use vacuum counters very effectively. Besides, most nursery container sowings are also using vacuum sowing devices.

Germination temperature and light

Although ISTA rules prescribe alternating temperatures of 20-30^{0}C ±1^{0}C for germination of most of the tree and shrub species with the lower temperature for 16 hours in darkness and the higher temperature for 8 hours in light . The changeovers from one temperature to the other is preferably lasting one hour or less but gradual changeover lasting three hours may be satisfactory. These requirements are difficult to meet as most of the commercial germinators cannot meet that specifications except those expensive germinators. Actually, the effect of constant temperature of 25^{0}C is very close to the alternating temperatures of 20-30^{0}C. This is especially true for moist chilled dormant seeds which can germinate under a wider temperature range after moist chilling treatment. The light source can be artificial or day-light.

Test period and monitoring germination progress (germination counts)

For tree and shrub species, ISTA prescriptions require the first germination count to be made on the 3rd to the 14th day depending upon species and the final count on the last day of the test period which ranges from 10 days (for poplars) to 56 days (for ashes). However, for most coniferous seeds, germination does not take place until 14th day except for the non-dormant seeds such as poplars, willows, jack pine, red pine. Therefore, the first count should be made on the 14th day instead of the 7th day.

If rate of seed germination is desired, more frequent counts are required (every day, every other day or three times a week). However, ISTA has not been able to incorporate vigour testing into the rules after nearly 36 years of research due to its complexity (Hampton and Coolbear 1990). Nevertheless, various vigour testing methods had been developed and widely used to measure rate of germination or storage potential. We must keep in mind that the information on seed vigour or rate of germination has to be simple and applicable to nursery operations. I would prefer the measurement of germination energy by assessing the cumulative germination percentage on the mid-term of the germination period (for most coniferous species 12-14 days after sowing). This information combined with the total germination can be used for determining whether a seedlot can be sown single seed per cavity.

Germination criteria

ISTA (1996) rules define 'germination of seed' in a laboratory test as "the emergence and development of the seedling to a stage where the aspect of its essential features indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil". This definition had not been applied to tree and shrub seeds until 1983 when Dr. Earl Belcher, the then Chairman of the Tree and shrub Seed Committee, recommended the adoption of the same germination criteria for tree and shrub seeds.

Prior to 1983, the germination criteria for tree and shrub seeds was the so-called '4 X' rule which defines seed as germinated when its emerging embryo is four times the length of the seed. The definition is rather loose as it was not clear whether it means the length of radicle and hypocotyl or just that of the radicle. In my earlier studies on the subject of seed germination criteria for red pine and white spruce, I found that seed has to germinate to certain stage where radicle, hypocotyl and cotyledons are developed normally and visible before it can be considered germinated. Only seeds germinated to such a stage and beyond under laboratory conditions were significantly correlated with nursery emergence (Wang 1973, 1976). Alberta has been using such germination criteria successfully for its nursery seedling production at Smoky Lake, AB.

Interpreting laboratory germination test results

On the ISTA certificate laboratory germination test results are reported on total normal and abnormal germination percentages, and ungerminated seeds. It also requires reporting the germination test results of pretreated seeds and the treatments involved if they are dormant. When a high percentage of viable seeds is found in the ungerminated seeds, it could indicate dormancy in the seedlot requiring moist chilling treatment. In contrast, when the moist chilled seeds germinated poorer than the non-chilled seeds, it could suggest the low vigour of or some injury to the seedlot. In such cases, moist chilling treatment should not be recommended or the use of such a seedlot should be reconsidered. This is where a mid-term germination energy measurement might be valuable for selecting vigorous seedlots in seedling production. Such a germination energy measurement along with other suggestions for interpreting tree seed germination tests was also recommended by Gosling and Peace (1990). I have some personal views about the cutting or viability tests of ungerminated seeds at the end of germination test period. Unless there is a great percentage of ungerminated seeds which could indicate improper treatment effects or injury of seeds sustained during handling or processing, I consider the effort made to determine those ungerminated seeds is unwarranted. Furthermore, it is difficult for the seed users to apply such information of seeds germinated with low vigour (less than full seedling development by the ISTA germination criteria) and the ungerminated viable seeds. I consider that if the seeds could not germinate under close to optimum conditions, there is little or no chance to them to be able to germinate under more stress nursery conditions.

Random arrangements of replications and treatments

In order for a completely blind test of germinability, different replicates and treatments of different seedlots are randomly arranged in different germination boxes(Wang and Downie 1991). The boxes are in turn arranged randomly on different shelves of the germinators. The boxes are changed around from time to time during the interim germination counts.

Electronic data collection

Each germination count for each test or study experiment conducted is entered manually, in a standardized format, into a file on a microcomputer. At the former National Tree Seed Centre, this file is a simple text file on a Radio Shack TRS 80, model 100 portable computer. The collected data file is uploaded to a mainframe computer. This procedure is standardized for most computers and requires but a jack compatible with the microcomputer and connected to the mainframe. Once all the collected data are uploaded to the mainframe, they are sequestered into a single large data file for various analyses required (detailed description of the procedure can be found in an unpublished report entitled " Integrated system for data collection, compilation and analysis for seed science" by Ben Wang and Bruce Downie, September 1991, National Tree Seed Centre, Petawawa National Forestry Institute). Today the data file can be uploaded to a PC for different analyses required using SAS software program.

The above comments and discussion are based on my nearly 30 years of observations and experience in tree seed testing and research, and I hope they will serve some useful purpose for seed analysts, nursery managers and seed researchers.

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I was recently sent a copy of the following publication from one of our regular contributors, Tom Noland. This is likely to be very useful for those involved in forest tree seed viability testing.

Noland, T. L., Mohammed, G. H. and Seymour, N. H. 1999. Using the Fluorescein Diacetate Staining Method to Estimate Seed Viability of Jack Pine, Black Spruce, and White Spruce. Ont. For. Res. Inst., Sault Ste. Marie, ON, For. Res. Note No. 58. 4pp.

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Short-term storage of untreated and pretreated seeds of *Leucaena leucocephala*

Summary

Untreated seeds of *Leucaena leucocephala* were 90% viable but only exhibited a germination capacity of 26%. Germination capacity was stimulated to an average of 74% by chipping, and suitable durations of boiling (100°C) or hot (90, 80, 70°C) water. Pretreated seeds were stored at 8% moisture content (fresh weight basis) and 4°C for 4, 8, 16 and 32 weeks and there was no deterioration in either viability or germination capacity.

Introduction

There is very little literature on the storage of pretreated tropical legume tree seeds and virtually all is confined to Acacia species. Osborn and Osborn (1931), Harding (1940), Moffett (1952), Isikawa (1965) and Sherry (1971) dried seeds of *Acacia mearnsii, A. mollissima and A. decurrens* after a boiling water pretreatment and ascertained that they could be safely stored for up to one year. Goo et al (1979) reported that seeds of *A. mearnsii* deteriorated over the course of 17 years storage.

The only information for non-Acacia species is that of Natarajan and Rai (1987) who stored acid pretreated *Leucaena leucocephala* seed for up to 12 months and concluded that 'acid scarification depressed germination during storage'. However, the basis for this statement was that the germination capacity of acid scarified seed dropped from 100% to 94% in contrast to non-scarified seed which only dropped to 98%.

In this paper we investigate the storage of untreated seeds of *L. leucocephala*, seeds pretreated by chipping and seeds pretreated by 8 of the 'best' boiling/hot water pretreatments reported by Gosling, Samuel and Jones (1995). Seeds were stored for up to 32 weeks at +4°C and 8 % moisture content.

Materials and Methods

Seeds of *Leucaena leucocephala* (Identity number [ID] 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) for 8 years.

Seed pretreatments

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

The eight best boiling/hot water pretreatments were selected from the results of Gosling et al (1995). The selection criteria were as follows. Firstly, that they had given as good a germination capacity as chipping. Secondly, they represented as wide a range of effective pretreatment temperatures as possible (70-100°C). Thirdly, they spanned as wide a range of effective durations as possible (7.5s-1h). The exact combinations of pretreatment temperatures and durations are recorded on the x-axes of Figures 1, 2 and 3.

Bulk seed pretreatment at different water temperatures was achieved by immersing 1 volume of seeds (secured in a nylon bag) in 10 volumes of water in a thermostatically controlled water bath. 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.

Seed germination

Germination tests were carried out after storage intervals of 0, 4, 8, 16, and 32 weeks. Four replicates of 50 seeds were sown on moist filter paper and incubated at an alternating 20/30°C (16h at 20°C in the dark followed by 8h at 30°C when the seeds were illuminated with c.11 Wm-2 light from warm white fluorescent tubes). 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 (1985)

according to Jones and Gosling (1994). mgt is equivalent to the average time taken for an average seed to germinate.

Storage of untreated and pretreated *L. leucocephala* seeds

Untreated, chipped and boiling/hot water bulk pretreated seeds of *L. leucocephala* (ID 44/88) were stored at 8% mc and 4°C in airtight polythene bags for 0, 4, 8, 16 and 32 weeks.

Two principal factors affect the longevity of seeds in storage - storage temperature and seed moisture content. Three of the selected boiling/hot water, bulk pretreatments led to a rise in mc from the initial 8%. The mc of seeds pretreated for 8m @ 80°C, 16m @ 70°C and 1h @ 70°C rose to 9, 9 and 13 %mc respectively. Therefore to avoid the confounding influence of storing seeds from different treatments at different mc's, these seeds were redried to 8% mc before storage by blotting with filter paper and incubating in a desiccator over silica gel. Seed with the highest mc took 4h to redry to 8% mc, therefore all treatments were put into storage at the same mc and on the same day that they were pretreated.

Statistical analysis

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

Results and discussion

Figure 1 shows that the viability of untreated and pretreated seeds was consistent between treatments and storage intervals. Statistical analysis of the transformed data confirmed that none of the differences were significant, therefore storage for up to 32 weeks did not lead to any deterioration in viability.

Figure 2 shows the effects of pretreatment temperatures and durations, plus the subsequent influence of storage on germination capacity. It is clear from this and statistical analysis of the transformed data that the germination capacity of untreated seeds was significantly lower than any of the pretreated seeds, and that all pretreatments were equally effective. In addition, germination capacity did not decrease after any of the storage intervals.

Perhaps the most interesting effect of storage is seen on germination rate expressed by mgt in Figure 3. It is clear for nearly all treatments except chipping that mgt after 32 weeks storage was much lower than at the outset. In fact the average mgt immediately after pretreatment (week 0) was 16 days, and after 32 weeks storage this was reduced to 13 days. There is even a hint from Figure 3 that this could be part of a trend, although statistical analysis did not confirm this. Osborn and Osborn (1931) noted a similar 'startling' increase in germination rate for *A. mearnsii* stored over 11 months. They offered no explanation for this phenomenon, but the same observation for seeds of *L. leucocephala* means that the response may be common. Clearly it would be desirable to confirm this statistically, and determine how long the improvement to germination rate continued during storage. This was not possible in the 32 weeks available on this project.

There is convincing evidence for a number of temperate tree species, that an increase in germination rate (decrease in mgt) under optimal germination conditions is indicative of seeds acquiring an ability to germinate more readily over a wider range of conditions (Gosling and Peace, 1990). The short term survival of *L. leucaena* seeds above, coupled with the changes in mgt therefore raise the interesting possibility that not only may pretreated multipurpose legume tree seeds resist deterioration, but that their subsequent performance may improve with time.

Figure 1. Viability of untreated and pretreated *Leucaena leucocephala* during storage for up to 32 weeks at 4°C

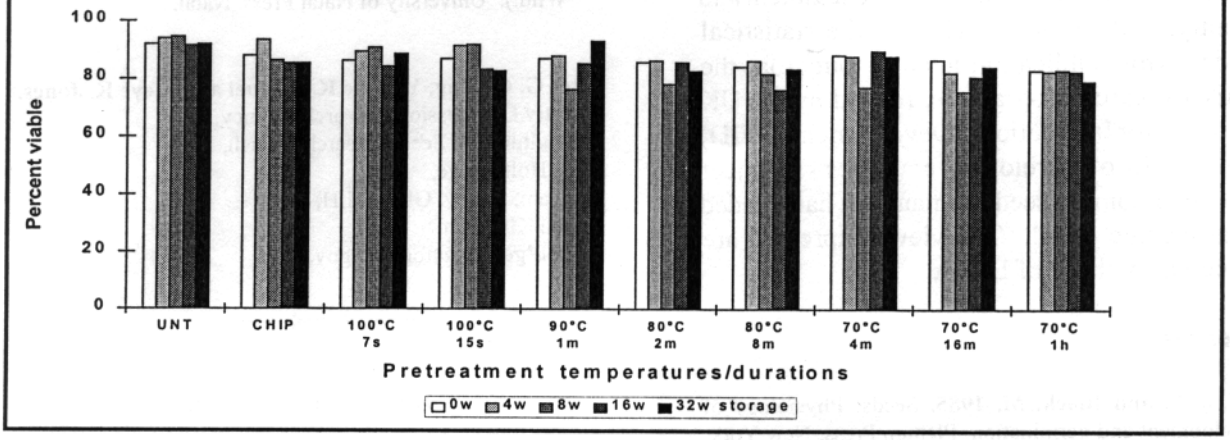


Figure 2. Germination capacity at 20/30°C of untreated and pretreated *Leucaena leucocephala* during storage for up to 32 weeks at 4°C

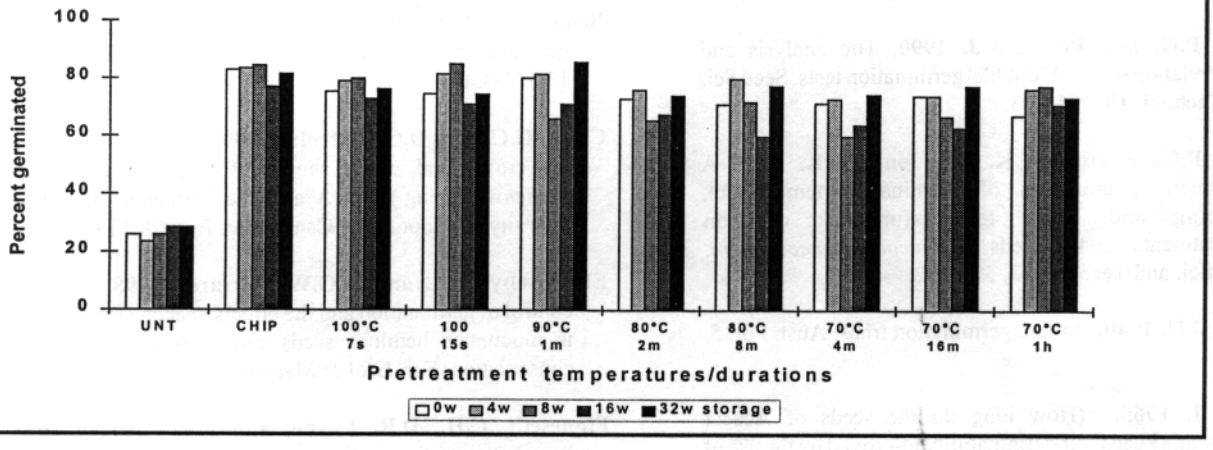
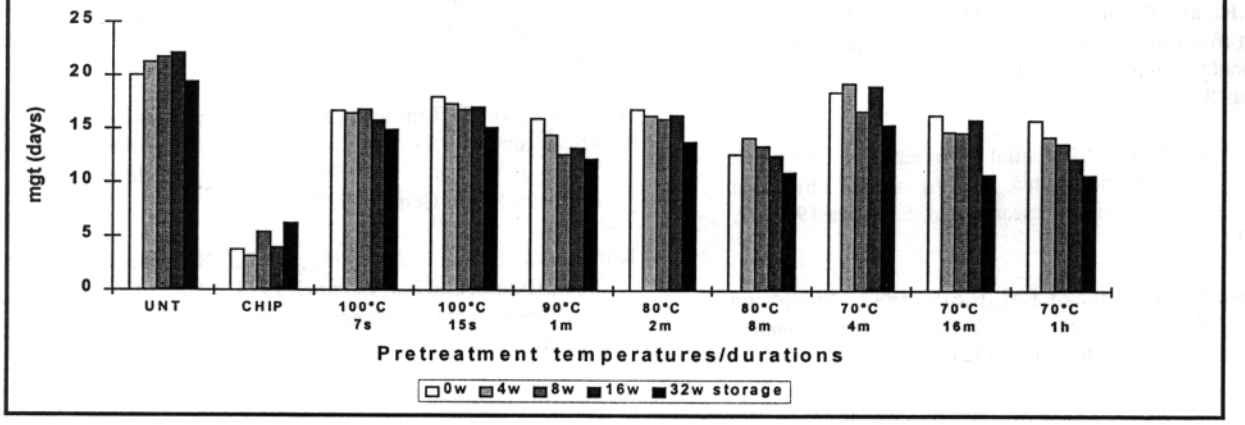


Figure 3. Germination rate at 20/30°C of untreated and pretreated *Leucaena leucocephala* during storage for up to 32 weeks at 4°C



Acknowledgements

We would like to thank Dr Elizabeth Major, Mrs Corinne Baker and Ms Sarah Packwood for carrying out much of the laboratory assessment and data entry, and Mr Jon Taylor for the statistical analysis. This publication is an output from the Forestry Research Programme, funded by the UK Department for International Development (DFID) for the benefit of developing countries - 'Project R5649 - overcoming seed dormancy in hardseeded tropical legume trees'. The views expressed are not necessarily those of DFID.

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