SUCCESSFUL STORAGE OF PRETREATED, READY-TO-SOW SEEDS OF *LEUCAENA LEUCOCEPHALA*

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GOSLING, P. G., SAMUEL, Y. K. & JONES, S. K. 2002. Successful storage of pretreated, ready-to-sow seeds of *Leucaena leucocephala*. Untreated seeds of *Leucaena leucocephala* exhibited 26% germination capacity and 90% viability. 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. Untreated and pretreated seeds were then stored at 8% moisture content (fresh weight basis) and 4 °C for 4, 8, 16, 32 and 64 weeks. Neither group (untreated or pretreated) showed any loss of viability or decrease in germination capacity over these storage periods and there was no change in germination speed (measured as "mean germination time", mgt) amongst untreated or chipped seeds. However, for all boiling/hot water pretreatments, not only were the beneficial effects of dormancy breakage retained throughout storage, but germination speed improved with increasing storage interval. The rate of improvement to mgt was described by a quadratic function. Boiling/hot water pretreated seeds of *L. leucocephala* not only resisted deterioration over 64 weeks storage, but their subsequent performance appeared to improve.

Key words: *Leucaena leucocephala* - seeds - pretreatment - storage - germination capacity - germination rate

GOSLING, P. G., SAMUEL, Y. K. & JONES, S. K. 2002. Kejayaan penyimpanan biji benih *Leucaena leucocephala* yang diprarawat dan sedia untuk ditanam. Biji benih *Leucaena leucocephala* yang tidak dirawat mempamerkan 26% keupayaan percambahan dan 90% kebolehhidupan. Keupayaan percambahan dirangsang kepada purata 74% melalui penyerpaian dan tempoh rendaman yang sesuai dalam air mendidih (100 °C) atau air panas (90, 80, 70 °C). Biji benih yang tidak dirawat atau yang diprarawat kemudiannya disimpan pada kandungan kelembapan 8% (berdasarkan berat segar) dan 4 °C selama 4, 8, 16, 32 dan 64 minggu. Kedua-dua kumpulan (tidak dirawat atau prarawat) tidak menunjukkan kehilangan kebolehhidupan atau pengurangan keupayaan percambahan pada tempoh penyimpanan ini. Di samping itu, tiada perubahan dalam kepantasan percambahan (disukat sebagai "masa percambahan min", mgt) pada biji benih yang tidak dirawat atau biji benih yang diserpai. Bagaimanapun, bagi biji benih yang diprarawat dengan air mendidih/air panas, bukan sekadar faedah pecahnya kedormanan dikekalkan di sepanjang tempoh penyimpanan, tetapi kelajuan percambahan juga meningkat dengan meningkatnya selang penyimpanan. Kadar peningkatan bagi mgt diterangkan melalui fungsi kuadrat. Biji benih *L. leucocephala* yang diprarawat dengan air mendidih/air panas bukan hanya menahan kemerosotan di sepanjang tempoh penyimpanan selama 64 minggu, tetapi prestasinya selepas itu didapati meningkat.

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Introduction

Legume trees are extensively planted in the tropics for a very wide range of purposes. They provide fuel-wood, sticks and poles for buildings and fences, and forage. Additional benefits include soil stabilisation, atmospheric nitrogen fixation, shade and shelter (Evans 1992, Nair & Muschler 1993). However, plant supply of these so called "multipurpose tropical legume trees" is rarely reliable. This is mainly because the seeds possess thick seed-coats which prevent or, at the very least, significantly retard germination. To stimulate germination, the hard seed-coats must be removed, punctured, abraded, split or softened–without damaging the embryo within. However, so-called "pretreatments" carried out at the nursery are not always successful. A helpful solution would be the development of a pretreatment method which could not only be successfully applied to large quantities of seeds, but would also allow their subsequent storage so that seeds pretreated before storage would be "ready-to-sow".

Despite considerable scientific, technical and anecdotal literature on the pretreatment and germination of many tropical legume tree seeds (Doran *et al.* 1983, Willan 1985, Cavanagh 1987, Hughes 1998), there is very little on their subsequent storage, and virtually all is confined to *Acacia* species. For example, Osborn & 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. Danthu *et al.* (1996) showed that acid-scarified seeds of *A. raddiana* (*A. tortilis* subsp. *raddiana* var. *raddiana*) and *A. albida* (or *Faidherbia albida*) maintained full viability throughout three years "cold chamber" storage, but those under ambient conditions (in Senegal), died within 18 and 8 months respectively. 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 & 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%.

We investigated the storage of untreated seeds of *L. leucocephala*, seeds pretreated by chipping and seeds pretreated using eight of the most effective boiling/hot water pretreatments reported by Gosling *et al.* (1995). Seeds were stored for up to 64 weeks at ± 4 °C and 8% moisture content.

Materials and methods

Seeds of *L. leucocephala* 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% (fresh weight basis) for eight years.

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. This is to avoid damaging the radical.

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 the widest possible range of effective pretreatment temperatures (70 to 100 °C). Thirdly, they spanned as wide a range of effective durations as possible (7.5 s to 1 hour). The exact combinations of pretreatment temperatures and durations are shown on the *x*-axes of Figures 1, 2 and 3 and in the legend to Figure 4.

Bulk seed pretreatment at different water temperatures was carried out by immersing one 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 min.

Seed germination and viability

Germination tests were carried out after storage intervals of 0, 4, 8, 16, 32 and 64 weeks. Four replicates of 50 seeds were sown on moist filter paper and incubated at an alternating 20/30 °C (16 hours at 20 °C in the dark followed by 8 hours at 30 °C when the seeds were illuminated with c.11 Wm^{-2} light from warm white fluorescent tubes). Seeds were considered germinated when the emerged embryo showed normal development at three times the length of the seed. Germination was assessed every Monday, Wednesday and Friday. Germination capacity was reached after 42 days. The assessment of abnormal seedlings and ungerminated seeds was made according to the ISTA rules (Anonymous 1993). Viability was measured as the combined percentage of "normal germinants + abnormal germinants + fresh seed (or if fresh seed exceeded 5% then tetrazolium-stained fresh seed)".

Mean germination time (mgt) is a common method for expressing germination rate as a single figure; it is equivalent to the average time taken for an average seed to germinate. In this study it was calculated using a modification of Bewley & Black (1985), according to Jones & Gosling (1994).

The equation used was

$$mgt_{pt} = mgt0_{pt} + b_1X + b_2X^2$$

where,

mgt_{pt} = mgt for a particular pretreatment,

$mgt0_{pt}$ = mgt at 0 weeks storage for a particular pretreatment (17.564 d for 100 °C and 7 s; 18.009 d for 100 °C and 15 s; 15.119 d for 90 °C and 1 min; 16.902 d for 80 °C and 2 min; 13.927 d for 80 °C and 8 min; 18.884 d for 70 °C and 4 min; 15.819 d for 70 °C and 16 min; 14.642 d for 70 °C and 1 hour)

b_1 = - 0.1256 (SE = 0.00225);
b_2 = 0.000965 (SE = 0.000336) and
X = storage duration (weeks)

Seed moisture content determination

Seed moisture content was determined in accordance with the ISTA Rules (Anonymous 1993) by drying samples at 103 ± 2 °C for 17 ± 1 hour. Moisture content was expressed as a percentage of the fresh weight of the seeds.

Storage of untreated and pretreated L. leucocephala *seeds*

Untreated, chipped and boiling/hot water bulk pretreated seeds of *L. leucocephala* were stored at 8% moisture content and 4 °C in airtight polythene bags for 0, 4, 8, 16, 32 and 64 weeks.

Two principal factors affect the longevity of seeds in storage, namely, storage temperature and seed moisture content (Harrington 1972). Three of the selected boiling/hot water, bulk pretreatments led to a rise in moisture content from the initial 8%. The moisture content of seeds pretreated for 8 min at 80 °C, 16 min at 70 °C and 1 hour at 70 °C rose to 9, 9 and 13% respectively. Therefore, to avoid the confounding influence of storing seeds from different treatments at different moisture contents, these seeds were redried to 8% moisture content before storage by blotting with filter paper and incubating in a desiccator over silica gel. Seed with the highest moisture content took 4 hours to redry to 8% moisture content. All treatments were put into storage at the same moisture content and on the same day that they were pretreated.

Statistical analysis

Angular transformation was applied to all percentage data to homogenise variances prior to analysis. The effects of different pretreatments and length of storage on viability and germination capacity were tested by analysis of variance (ANOVA). The effect of storage duration and pretreatment on mgt was investigated by fitting a sequence of regression models.

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 differences were not significant. Therefore, storage for up to 64 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 evident that the germination capacity of untreated seeds was significantly lower than any of the pretreated seeds, irrespective of storage duration ($p \leq 0.001$). Statistical analysis of the transformed data for all pretreatment and storage durations indicated that all pretreatments were equally effective (average germination capacity 74%), and that germination capacity did not decrease after any of the storage intervals ($p \leq 0.001$). The beneficial effects of pretreatment on germination capacity were therefore retained for at least 64 weeks of storage at 8% moisture content and 4 °C. These observations are in accordance with those of Osborn and Osborn (1931), Harding (1940), Moffett (1952), Isikawa (1965), Sherry (1971) and Danthu *et al.* (1996) for pretreated and stored seeds of *A. mearnsii, A. mollissima, A. decurrens, A. raddiana* and *A. albida*. However, it is interesting to note that they are in direct contrast to those of Jones *et al.* (1998) who reported that the storage of prechilled Sitka spruce (*Picea sitchensis*) seeds led to dormancy reimposition. One explanation for this is that *Acacia* and *Leucaena* seeds exhibit a coat-imposed dormancy which probably responds physically to pretreatment, and this change is permanent, whereas the response of spruce seeds (which probably respond physiologically to a moist prechill) is reversible.

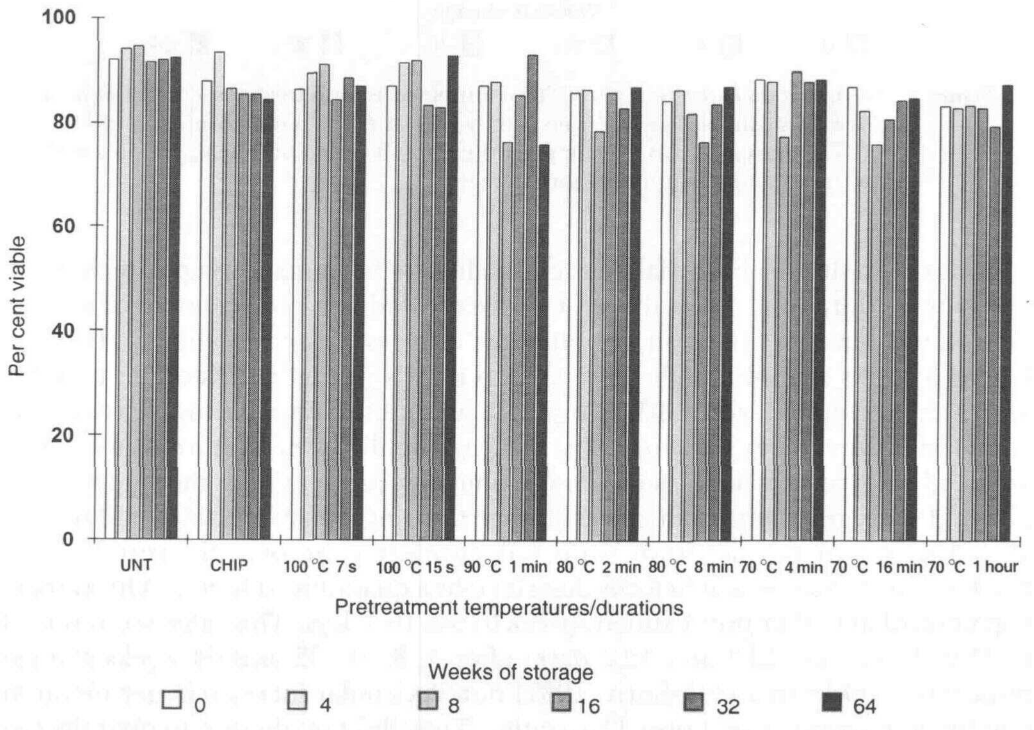

Figure 1 Viability of untreated and pretreated seeds of *Leucaena leucocephala* during storage for up to 64 weeks at 8% moisture content and 4 °C. (UNT - untreated; CHIP - chipped; then hot/boiling water temperatures and pretreatment durations before storage)

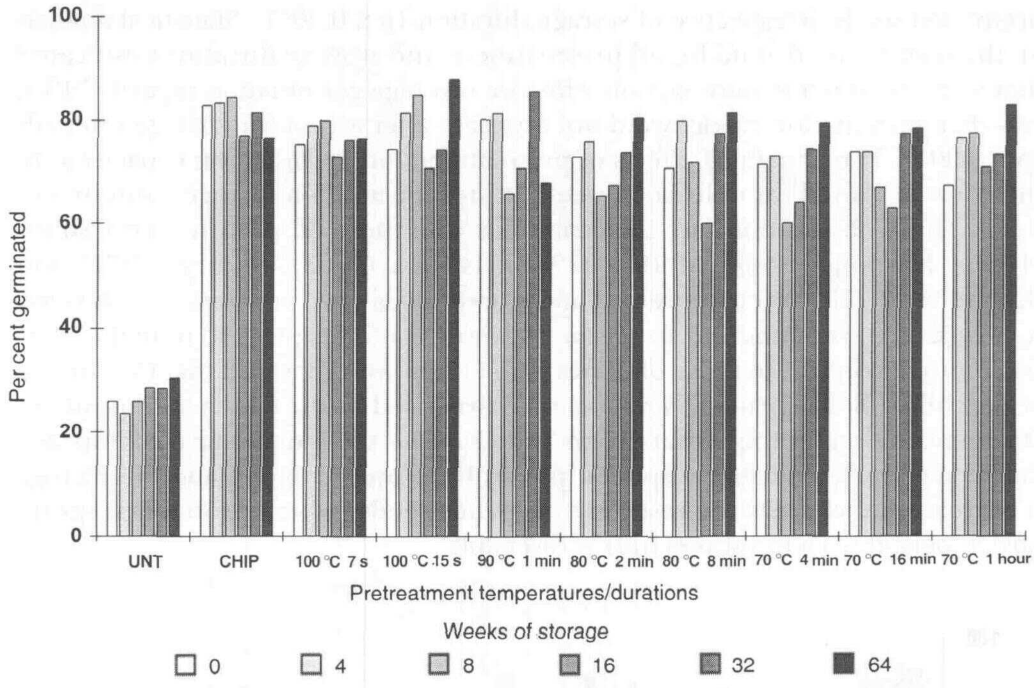

Figure 2 Germination capacity at 20/30 °C of untreated and pretreated seeds of *Leucaena leucocephala* during storage for up to 64 weeks at 8% moisture content and 4 °C. (UNT - untreated; CHIP - chipped; then hot/boiling water temperatures and pretreatment durations before storage)

It has been shown above that neither viability nor germination capacity changed with storage duration. It was therefore not expected that pretreatment or storage time would have an effect on germination rate (expressed by mgt). However, Figure 3 shows that although there are no trends in mgt with storage time for untreated or chipped seeds, all boiling/hot water pretreatments showed a decrease in mgt with storage time. Figures 4(a) and (b), highlight this feature on a linear *x*-axis, and a regression analysis of storage time and pretreatment method showed that mgt was significantly reduced as storage time increased ($p \leq 0.001$). The rate of reduction in germination time was consistent across all pretreatment combinations and was satisfactorily described by a quadratic function. The average mgt immediately after pretreatment (week 0) was 16.4 days. This value was reduced to 15.9, 15.4, 14.6, 13.3 and 12.2 days after 4, 8, 16, 32 and 64 weeks storage respectively. Osborn and Osborn (1931) noted a similar increase in germination rate for *A. mearnsii* stored over 11 months. They did not attempt to quantify the response, nor offer an explanation for this phenomenon, but the same observation for seeds of *L. leucocephala* may suggest that the response is common amongst tropical legume tree seeds with physical dormancy. Boiling or hot water treatments create small fractures in the seed coats of individual seeds. It is possible that increasing the duration of storage may allow these fractures to enlarge. Hence stored seeds may imbibe faster on subsequent exposure to water and this could

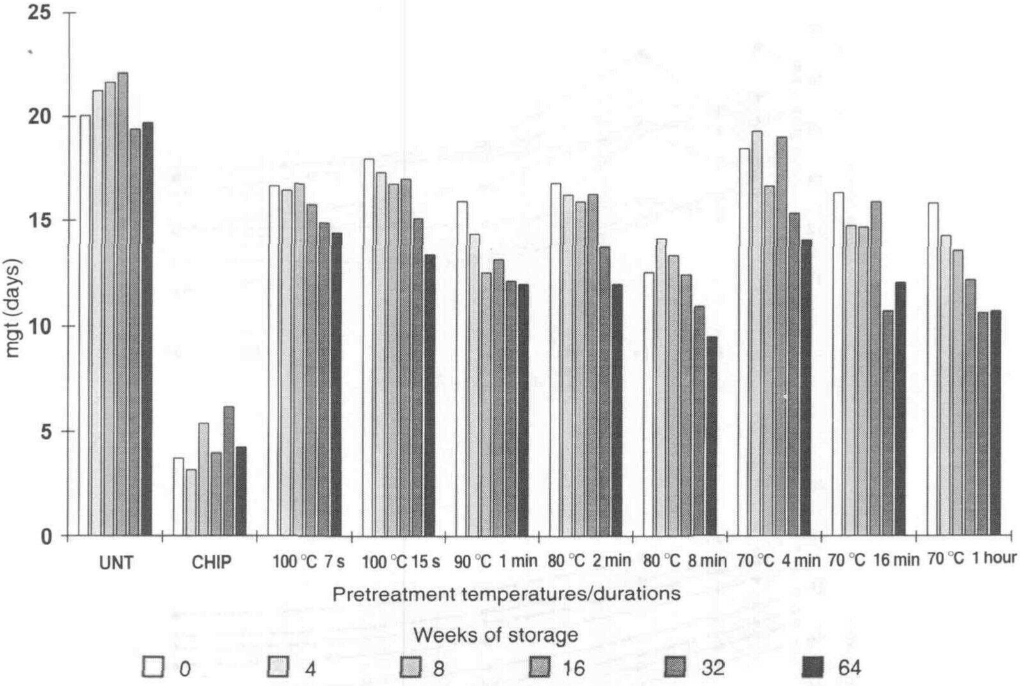

Figure 3 Germination rate (mean germination time) at 20/30 °C of untreated and pretreated seeds of *Leucaena leucocephala* during storage for up to 64 weeks at 8% moisture content and 4 °C

accelerate germination speed. Clearly it would be desirable to determine experimentally how long the improvements to germination rate continued during storage. Unfortunately, the project did not continue past 64 weeks. However, extrapolation of the mgt results for seeds pretreated by boiling or hot water suggests that their germination rate would never approach the germination speed of chipped seeds.

There is convincing evidence for a number of temperate tree species showing 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 & Peace 1990). The short-term survival of *L. leucocephala* seeds, 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.

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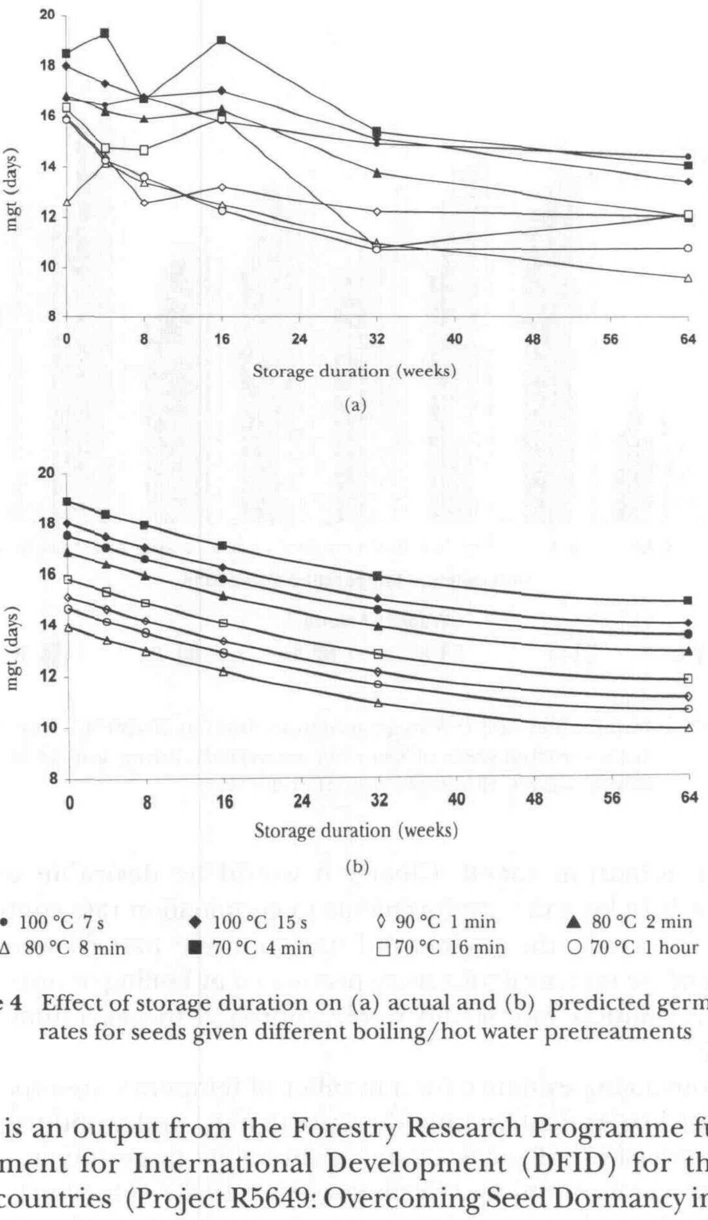

Figure 4 Effect of storage duration on (a) actual and (b) predicted germination rates for seeds given different boiling/hot water pretreatments

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