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# Effects of accelerated ageing on viability, leachate exudation, and fatty acid content of *Dalbergia sissoo* Roxb. seeds

R. C. THAPLIYAL[1] and K. F. CONNOR[2]*

[1] Seed Testing Laboratory, Silviculture Division, Forest Research Institute, P.O. New Forest, Dehra Dun-248006, India
[2] USDA Forest Service, Forestry Sciences Laboratory, P.O. Box 928, Starkville, MS 39760

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## Summary

Accelerated ageing of seeds of *Dalbergia sissoo* Roxb., a multi-purpose tropical legume tree, was effective as a vigour test only at temperatures in excess of 43 C for 72 h. Increased leakage of solutes accompanied the decrease in viability, but there was no relationship between seed size and conductivity. Analyses of *D. sissoo* lipids indicated that palmitic acid was the most common saturated fatty acid and linoleic acid the most common unsaturated fatty acid. While total percentages of saturated and unsaturated fatty acids varied little throughout the course of the experiment, quantities of all individual fatty acids decreased during the ageing process.

## Introduction

A chain of physiologial events begins in seeds long before the cessation of viability. It starts with the degradation of membranes and passes through energy synthesis mechanism impairment. Decline in respiration, biosynthesis, germination rate, storability, rate of growth and development, uniformity, plant resistance, yield, and field emergence occur, as does an increase in the number of abnormal seedlings (Delouche and Baskin 1973). Quantitative and qualitative changes in carbohydrates, lipids and proteins also provide information about metabolic changes associated with deterioration during storage (Anderson 1973).

All these events can take place over a period as short as a few weeks or as long as several years. In order to obtain information on seed vigour in a timely manner, the accelerated ageing technique is commonly used, through which changes in the seed at the cellular level during long-term storage can be simulated within a comparatively short period of time by exposing seeds to increased temperatures (40–45 °C) and a high relative humidity (Delouche, Rushing and Baskin, 1968; Abdul-Baki, 1969; Delouche and Baskin, 1973). Accelerated ageing is an accepted vigour test for many agricultural species (AOSA, 1983) and for some temperate forest tree species (Bonner, 1984; Blanche, Elam and Hodges, 1990; Marquez-Millano, Elam, and Blanche, 1991).

Research on deterioration of many tropical tree seeds under accelerated ageing condi-

* Corresponding author

tions has not been done. In this paper we explore whether or not the technique can be applied to *Dalbergia sissoo* Roxb. seeds. In addition, we determine if significant changes in leachate conductivity and fatty acid composition occur in aged seeds. *Dalbergia*, a valuable multipurpose legume tree, occurs naturally over a wide range of climatic conditions, from the valleys in the outer Himalayas where severe winter frost is a regular climatic feature to the heat-stressed upper Gangetic plains. *Dalbergia* has also been planted in the states of Rajasthan, Haryana, and Punjab, where it has adapted well to summer temperatures which can exceed 47 °C.

## Materials and methods

In December, 1994, seeds of *D. sissoo* were collected from two locations in India (twenty trees per locality) and bulked. Seed lot A was from a natural forest located on an ideal exposed river bed site, while seed lot B was collected from a planted understory of *Dalbergia* trees growing in a natural forest of *Shorea robusta*. The seeds were manually extracted from the pods and treated with pesticides and insecticides for quarantine purposes before being brought to the United States.

### *Accelerated ageing procedure*

Seeds were washed throughly in distilled water and blotted dry. After the initial moisture content was determined according to the International Seed Testing Association Rules (ISTA, 1993), the seeds were placed on wire mesh holders inside 'Stults® Accelerated Aging' plastic boxes (14 cm × 14 cm × 5 cm) containing 70 ml of distilled water. The containers were then randomly placed inside 'Stults® Accelerated Aging' cabinets, and the seeds were subjected to four different temperatures – 39 °C, 41 °C, 43 °C and 45 °C – and nearly 100% relative humidity. Four replications of fifty seeds for the germination test and a weighed quantity of seed (about 500 mg) for fatty acid determination were withdrawn at intervals of 48, 72 and 96 h. The seeds withdrawn for the germination tests were first used for the determination of leachate conductivity. Seeds not subjected to accelerated ageing were used as controls.

### *Germination tests*

An initial germination test was conducted at 20 °C/30 °C and 8/16 h day/night cycles. Since the germination rate using this temperature and light program was low, all tests in this experiment were carried out at a constant 30 °C and 12 h day/night cycles. Seeds were considered germinated when the cotyledons emerged fully from the seedcoat.

### *Leachate conductivity measurements*

After each accelerated ageing treatment, seeds were submerged in 40 ml of glass distilled water at room temperature. After 24 h, the seeds were removed, and electrical conductivity of the leachate water was determined using a YSI® model 32 single probe conductivity meter.

*Lipid extraction and fatty acid analysis*

A weighed quantity of seed (500 mg) was ground in liquid nitrogen and then stirred in hot isopropyl alcohol for 10 min (Fishwick and Wright 1977). The seed material was filtered out, the alcohol vacuum evaporated in a flask, and the seed pellet twice rinsed with 2:1 chloroform ($CHCl_3$)/methanol (MEOH). The pellet, rinse, contents of the vacuum flask, and 3 mg of an internal standard (heptadecanoic acid) were stirred in 30 ml of the 2:1 $CHCl_3$/MEOH for 30 min and then filtered. The supernatant was measured into a separation flask and shaken once with a 0.9% (w/v) sodium chloride (NaCl) solution measuring 0.2× its volume and twice with a 1:1 MEOH/0.9% (w/v) NaCl solution measuring 0.5× the original volume, in the manner of Folch, Lees and Sloane Stanley (1957). The bottom layer was drained into a flask, vacuum evaporated, and the lipids redissolved in 0.5 ml $CHCl_3$.

The fatty acids and 1 ml of a 14% (w/w) solution of boron trifluoride-MEOH (premixed) were pipetted into a reaction vial and heated in boiling water for 3 min (Metcalfe and Schmitz 1961). The reaction was stopped with 1 ml of distilled water, and 2 ml of petroleum ether were added to the vial. After shaking, the upper layer was pipetted into a vial and dried under a stream of $N_2$. The methyl esters were redissolved and stored in 0.45 ml of $CHCl_3$. Fifty µl of a second standard, nonadecanoic acid (1.5 mg/ml), were added just prior to sample analysis on a HP 5890 gas chromatograph equipped with a 30 m × 0.53 mm × 0.5 µ J & W megabore DB-23 column. The initial column temperature, 110°C, was held for 5.50 min and then increased at 30°C/min to 150°C and held for 20 min. Injector temperature was set at 200°C and detector temperature at 220°C. A petroleum ether soxhlet extraction was also performed on 1 g of freeze dried seed from each seed lot.

## Results and discussion

*Moisture content and germination*

The moisture content of the two seed lots varied somewhat initially; seed lot A had a moisture content of 10.1%, and seed lot B of 8.9%. The germination of control seeds of both lots A and B was very high, 97.5 and 95.5 respectively (Table 1). However, differences between lots were discernible following ageing; lot B was almost always characterized by poorer germination than lot A. After 48 h at 39, 41, and 43°C, germination of lot B was significantly lower than that of lot A ($p = 0.05$), which remained relatively unaffected by the increased temperature and humidity. However, as duration of the accelerated ageing treatment increased, the germination differences between the seed lots became non-significant ($p = 0.07$, 0.34 at 72 h and 96 h, respectively). Only at 45°C was there a sharp decline in the germination percentage of both the lots, which increased with time of ageing. This suggests that the best combination of time and temperature for accelerated ageing tests of *D. sissoo* seeds is 72 h at 43°C, and that the initial variation in moisture content was too small to affect the accelerated ageing procedure.

Increasing temperatures up to 43°C had almost no apparent adverse effect on the

Table 1. Effect of accelerated ageing on the germination of *Dalbergia sissoo* seed.

| Sample lot | Temp. (°C) | Hours aged 0* | 48 | 72 | 96 |
|---|---|---|---|---|---|
| A | 0 | 97.5 | – | – | – |
| | 39 | – | 91.0 | 92.0 | 89.0 |
| | 41 | – | 95.0 | 60.5 | 89.5 |
| | 43 | – | 93.0 | 62.0 | 70.0 |
| | 45 | – | 34.5 | 23.8 | 12.5 |
| B | 0 | 95.5 | – | – | – |
| | 39 | – | 73.0 | 89.0 | 79.5 |
| | 41 | – | 67.5 | 54.0 | 72.0 |
| | 43 | – | 71.5 | 43.5 | 75.0 |
| | 45 | – | 28.5 | 17.0 | 14.5 |

*Controls

emergence of radicles. Abnormalities began to develop later, however, with the radicle turning brown as the seedlings matured. The higher percentage of abnormal seedlings accounted for the low percentage of germination at temperatures below 43 °C, but at 45 °C there was a higher rate of decline in germination due to seed decay. The lower germination values after 72 h of accelerated ageing remain inexplicable, but do match a similar trend in the leachate conductivity data (Figure 1). On the whole, seeds of *D. sissoo* may be expected to tolerate high temperatures and humidity since these conditions prevail in its natural habitat between the time of seed dispersal and germination. Seeds

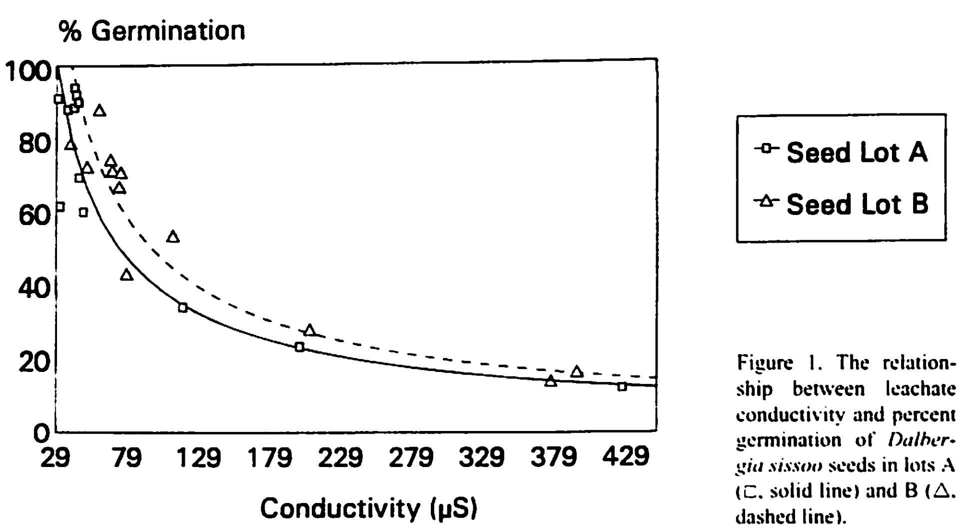


Figure 1. The relationship between leachate conductivity and percent germination of *Dalbergia sissoo* seeds in lots A (□, solid line) and B (△, dashed line).

are dispersed by the wind intermittently throughout the early summer and remain exposed on the ground in the hot summer months.

*Leachate conductivity*

Figure 2 shows the conductivity of leachates per gram fresh weight of seed. At all temperatures through 43 °C, the conductivity remained below 100 µS for all periods of ageing. Predictably, the conductivity values were generally higher for lot B than A. Leachate conductivity increased sharply at the 45 °C accelerated ageing temperature, and, as expected, values increased as ageing progressed from 48 h to 96 h. The importance of temperature treatment on leachate conductivity was fairly significant ($r > 0.75$) on both seed lots throughout the experiment (Table 2). However, low r values resulted when the effect of duration of the experiment on leachate conductivity was measured at 39, 41, and 43 °C; the exception was at 45 °C, when duration of the accelerated ageing treatment proved very important in both seed lots A and B ($r = 0.97$ and 0.82, respectively). There was no relationship between seed size and leachate conductivity; this has generally been the case with smaller seeded species (Vozzo and Bonner, 1985; Bonner and Vozzo, 1987).

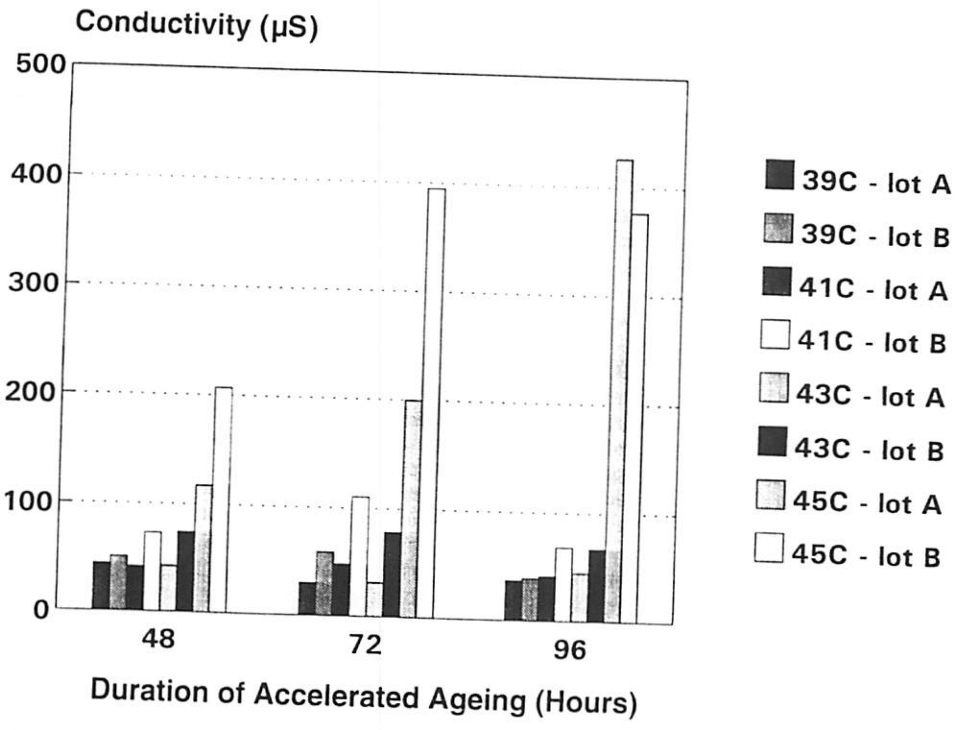


Figure 2. The effect of accelerated ageing on the leachate conductivity measurements of *Dalbergia sissoo* seed lots A and B. Values presented are µS/g fresh weight.

Table 2. Correlation coefficients (r) measuring the importance of temperature on leachate conductivity after 48, 72, and 96 h of accelerated ageing.

| Duration (h) | Seed lot A | Seed lot B |
|---|---|---|
| 48 | .764 | .850 |
| 72 | .776 | .799 |
| 96 | .785 | .817 |

*Lipid extraction and fatty acid analysis*

Soxhlet extractions of *D. sissoo* seed yielded a low amount of lipid, 0.0581 g/g dry weight. Analyses of lipids determined that the most common saturated fatty acid was 16:0 (palmitic acid, 19.5%), and the most common unsaturated fatty acid was 18:2 (linoleic acid, 43.9%). Quantities of individual fatty acids per gram of seed tissue are presented in Table 3. Overall, both temperature and duration of the accelerated ageing treatment had a significant effect on the seed fatty acid content (Table 4). The high negative correlations in seed lot A at 72h and 96h and in seed lot B at 96h indicated that significant reductions in all major fatty acids were occurring at both temperatures 41 °C and 45 °C after 3 days of accelerated ageing. Despite this overall reduction, the individual fatty acid makeup of the lipid varied little between treatments and seed lots (Table 5). In seed lot B, individual fatty acid percentages increased or decreased as little as 2% at all times and temperatures throughout the experiment; variation was 6% in seed lot A.

Table 3. Individual fatty acid contents (mg/g fresh weight) of *Dalbergia sissoo* seeds subjected to accelerated ageing at 41 °C and 45 °C. Values within a column followed by the letter 'a' are not significantly different from the control value at $p \leq 0.05$.

| Lot[1] | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 20:0 | Total |
|---|---|---|---|---|---|---|---|---|---|
| A-control | 0.01 | 0.10 | 3.91 | 0.22 | 1.89 | 3.54 | 8.92 | 1.17 | 19.76 |
| A-41-48 | 0.01a | 0.03a | 1.27b | 0.10a | 0.74a | 1.18a | 2.81a | 0.39a | 6.53 |
| A-41-72 | 0.01a | 0.04b | 1.51a | 0.10a | 0.86a | 1.52a | 4.00a | 0.52a | 8.56 |
| A-41-96 | 0.00a | 0.02a | 0.83b | 0.07a | 0.54a | 0.76b | 1.84b | 0.26a | 4.32 |
| A-45-48 | 0.01a | 0.05a | 2.44a | 0.08b | 1.22a | 2.25a | 5.73a | 0.77a | 12.55 |
| A-45-72 | 0.00a | 0.02a | 0.90b | 0.08a | 0.49a | 0.79b | 1.91b | 0.26a | 4.45 |
| A-45-96 | 0.00a | 0.00a | 0.07b | 0.00b | 0.05b | 0.07b | 0.12b | 0.01b | 0.32 |
| B-control | 0.01 | 0.08 | 4.09 | 0.16 | 2.05 | 3.47 | 9.06 | 1.30 | 20.22 |
| B-41-48 | 0.01a | 0.02b | 1.07b | 0.13a | 0.66b | 0.88b | 2.27b | 0.30b | 5.34 |
| B-41-72 | 0.00a | 0.04a | 1.18b | 0.21a | 0.74b | 0.99b | 2.67b | 0.36b | 6.19 |
| B-41-96 | 0.01a | 0.06a | 1.94b | 0.08b | 1.28b | 1.78b | 4.82b | 0.58b | 10.55 |
| B-45-48 | 0.00b | 0.05b | 1.89b | 0.11b | 1.03b | 1.56b | 4.25b | 0.58b | 9.47 |
| B-45-72 | 0.01a | 0.05a | 2.15a | 0.16a | 1.24a | 1.94a | 4.99a | 0.65a | 11.19 |
| B-45-96 | 0.00a | 0.04b | 1.93b | 0.01b | 1.12b | 1.79b | 4.70b | 0.63b | 10.22 |

[1] Lot = Lot A or B – accelerated ageing temperature – time of ageing.

Table 4. Correlation of amounts of individual fatty acids of two *Dalbergia sissoo* seed lots and temperature of accelerated ageing treatment. All correlation values are negative.

| Seed Lot | Duration | Palmitic 16:0 | Stearic 18:0 | Oleic 18:1 | Linoleic 18:2 | Arachidic 20:0 |
|---|---|---|---|---|---|---|
| A | 48h | .749 | .768 | .739 | .720 | .712 |
| | 72h | .993 | .994 | .995 | .994 | .994 |
| | 96h | .994 | .996 | .994 | .993 | .994 |
| B | 48h | .784 | .788 | .788 | .785 | .777 |
| | 72h | .710 | .647 | .654 | .706 | .736 |
| | 96h | .955 | .990 | .954 | .961 | .935 |

We conclude that the accelerated ageing technique works well on seeds of *D. sissoo*, despite the extremes of temperature found in the species' native environment. While temperatures up to 43 °C had no effect on the germination of vigourous seed, seeds did deteriorate when exposed to 45 °C and a high relative humidity. Interestingly, while initial viability was high for both seed lots, seeds from the more ideal site (A) almost always had a higher percent germination than those from site B. This suggests some connection between seed vigour and site quality and indicates that, when harvesting *Dalbergia* seeds for planting, superior nutrient-rich sites should be selected in order to obtain the best seed (Kearney and Bonner 1968; Owens and Blake 1985). This conclusion is reinforced by further germination studies on the seeds. After 9 months stor-

Table 5. Individual fatty acid contents (%) of *Dalbergia sissoo* seeds subjected to accelerated ageing at 41 °C and 45 °C. Values within a column followed by the letter 'a' are not significantly different from the control value at $p \leq 0.05$.

| Lot[1] | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 20:0 |
|---|---|---|---|---|---|---|---|---|
| A-control | 0.0 | 0.5 | 19.8 | 1.1 | 9.6 | 17.9 | 45.2 | 5.9 |
| A-41-48 | 0.10b | 0.5a | 19.7a | 1.6b | 11.3b | 18.0a | 43.0b | 5.9a |
| A-41-72 | 0.1a | 0.4a | 17.6b | 1.2a | 10.1b | 17.7a | 46.8b | 6.1b |
| A-41-96 | 0.1b | 0.7b | 18.5b | 1.7b | 12.5b | 17.9a | 42.7b | 5.9a |
| A-45-48 | 0.1a | 0.4b | 19.3a | 0.6b | 9.8a | 18.0a | 45.7b | 6.1b |
| A-45-72 | 0.1b | 0.5a | 19.9a | 1.9b | 11.1b | 17.8a | 43.0b | 5.8b |
| A-45-96 | 0.0b | 0.3a | 22.1b | 0.0b | 14.4b | 20.3b | 39.0b | 3.9a |
| B-control | 0.0 | 0.4 | 19.6 | 0.7 | 9.9 | 16.7 | 46.3 | 6.3 |
| B-41-48 | 0.1a | 0.4a | 19.9a | 2.4a | 12.4b | 16.5b | 42.7b | 5.7b |
| B-41-72 | 0.1a | 0.6a | 19.0b | 2.8a | 12.1b | 16.1a | 43.5a | 5.9a |
| B-41-96 | 0.1a | 0.5b | 18.1b | 0.7a | 12.2b | 17.0b | 45.9b | 5.5a |
| B-45-48 | 0.0a | 0.5b | 20.0a | 1.2a | 10.8b | 16.5b | 44.9b | 6.1b |
| B-45-72 | 0.1b | 0.5b | 19.1a | 1.5a | 11.0b | 17.2a | 44.6b | 5.8b |
| B-45-96 | 0.0a | 0.4b | 18.8b | 0.9a | 10.8b | 17.6b | 45.3a | 6.1a |

[1] Lot = Lot A or B – accelerated ageing temperature – time of ageing.

age at room temperature, viability of seed lot A was still very high (95.5%), while that of lot B had dropped to 38%. The percent of abnormal seedlings was 1% in lot A and 8% in lot B.

As expected, leachate conductivity was positively related to accelerated ageing temperature, indicating that increased permeability of the membranes is involved in the ageing process. However, a direct relationship between conductivity and percent germination was not evident. Changes in the fatty acid content of the aged seeds were also indicative of decreasing viability. There was generally a drop in total fatty acid content with time of ageing; however, the overall percent composition of the individual fatty acids making up the lipid component varied little throughout the course of the experiment.

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