EVALUATING THE RELATIVE STORABILITY OF IDS-TREATED AND UNTREATED *PINUS PATULA* SEEDS BY ACCELERATED AGEING

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DEMELASH, L., TIGABU, M. & ODÉN, P. C. 2004. Evaluating the relative storability of IDS-treated and untreated *Pinus patula* seeds by accelerated ageing. IDS (Incubation, Drying and Separation)-treated and untreated *Pinus patula* seeds were subjected to nine accelerated ageing regimes in 100% relative humidity at 41 °C to evaluate their relative storability. Germination capacity, mean germination time and leachate conductivity were measured before and after ageing treatments. All accelerated ageing treatments decreased the germination capacity (viability) and speed of germination (vigour). The reduction of viability and vigour was greater for untreated seeds. Seed moisture content and leachate conductivity also increased with ageing. The latter was significantly higher in untreated seeds. Leachate conductivity was negatively correlated with germination capacity and positively correlated with mean germination time for both seeds. Since IDS-treated seeds tolerated the stress induced by accelerated ageing better than untreated seeds, it can be concluded that IDS-treated seeds will have better storability. The strong positive correlation found between leachate conductivity and mean germination time indicates that leachate conductivity can be used as a quick test of vigour for *P. patula* seeds.

Key words: Leachate conductivity – germination – viability – vigour – seed quality – seed deterioration

DEMELASH, L., TIGABU, M. & ODÉN, P. C. 2004. Menilai penyimpanan relatif biji benih *Pinus patula* yang dirawat dan yang tak dirawat selepas pengumuran terpecut. Biji benih *Pinus patula* yang dirawat (penyeraman, pengeringan dan pengasingan) dan yang tak dirawat didedah kepada sembilan keadaan pengumuran terpecut pada 100% kelembapan relatif dan 41 °C untuk menilai penyimpanan relatif masing-masing. Keupayaan percambahan, purata masa percambahan dan kekonduksian larutan lesap disukat sebelum kajian dan selepasnya. Semua rawatan pengumuran terpecut mengurangkan keupayaan percambahan (kebolehidupan) dan kadar percambahan (kesuburan). Pengurangan dalam kebolehidupan dan kesuburan lebih tinggi pada biji benih yang tak dirawat. Kandungan lembapan biji benih dan kekonduksian larutan lesap bertambah dengan pengumuran. Biji benih yang tak dirawat menunjukkan kekonduksian larutan lesap yang lebih tinggi. Kekonduksian larutan lesap berkorelasi secara negatif dengan keupayaan percambahan dan berkorelasi secara positif dengan purata masa percambahan bagi kedua-dua jenis biji benih. Oleh kerana biji benih yang dirawat dapat menahan tekanan yang lebih daripada pengumuran terpecut berbanding biji benih yang tak dirawat, boleh diputuskan bahawa biji benih yang tak dirawat akan mempunyai nilai penyimpanan yang lebih baik. Korelasi positif di antara kekonduksian larutan lesap dengan purata masa percambahan menujukkan bahawa kekonduksian larutan lesap boleh digunakan sebagai ujian cepat untuk menilai kesuburan biji benih *P. patula*.

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Introduction

The storability of a seedlot is primarily determined by the vigour of the seed at maturity and level of deterioration at the time of storage. A reduction in storage potential is one of the specific consequences of seed deterioration and is highly influenced by its pre-storage history and the storage conditions (Roberts 1973). Seed deterioration occurs as a result of physiological and biochemical perturbations. As an imbibed seed deteriorates, energy synthesis mechanisms are impaired, respiration and biosynthesis activities are reduced, chromosomal aberration and DNA degradation occur, as well as changes in RNA and protein synthesis, enzymatic activities, food reserve and membrane alteration, which eventually reduce seed vigour, storability, germination capacity and emergency potential (Smith & Berjak 1995, Marcos-Filho & McDonald 1998). In addition, deteriorated seeds are susceptible to stress and would result in a large number of abnormal seedlings as well as poor plant development and yield (Delouche & Baskin 1973, Bewley & Black 1994, Kalpana & Madhava 1995). Major changes in chemical composition during deterioration of seeds are an increase in free fatty acids and inorganic phosphate, a decrease in neutral lipids, phospholipids, total soluble sugar, proteins and starch (Harrington 1973, Blanche *et al.* 1990, Jeng & Sung 1994, Madhava & Kalpana 1994, Taylor *et al.* 1995). The loss of the ability to produce plant hormones, i.e. gibberellic acid, cytokinins and ethylene necessary for triggering germination, is also a fundamental process during ageing of seeds (Harrington 1973). An extensive review on the mechanism and kinetics of seed ageing can be found in Walters (1998).

Loss of integrity and subsequent excessive exudation of organic and inorganic seed constituents from the membrane are usually the initial symptoms of seed deterioration (e.g. Basavarajappa *et al.* 1991). Several compounds including amino acids, organic acids, inorganic ions, sugars, phenolics and proteins can be detected as they leak from imbibing seeds. The quantity of exuded solutes is a diagnostic of seed quality and is the basis for electrolyte leakage assay for seed vigour testing (Thornton *et al.* 1990, Bonner 1991a, Sorensen *et al.* 1997, Thapliyal & Connor 1997, Rehman *et al.* 1999). Seeds that leak excessively are slow to complete germination and are considered to be more susceptible to disease and insect attack (Hartmann *et al.* 1997).

It is often not possible to consistently predict storability of a seedlot by a standard germination test prior to storage unless the difference in germination capacity among seedlots is initially large. Delouche and Baskin (1973) had shown the deficiencies of a germination test as a sole measure of physiological quality and storability of seedlots and further developed accelerated ageing techniques for predicting the relative storability of seedlots. These techniques involve the exposure of small seed samples from all lots to adverse levels of temperature (40–45 °C) and relative humidity (about 100%) for a period of 2–8 days or at 30 °C and 75% relative humidity for 2 to 24 weeks followed by regular germination tests. The assumption is that seedlots maintaining high germination during accelerated ageing will have good storability under normal conditions while those performing poorly will rapidly lose their viability and vigour during storage. In most cases, accelerated ageing and storage responses are closely associated. For

example, Vanangamudi *et al.* (2000) had used accelerated ageing to predict storability of neem (*Azadirachta indica*) and jamun seeds (*Syzygium cuminii*).

The relative storage potential of seedlots could be predicted with many other tests and measurements such as rate of respiration during early stage of germination, seedling growth rate, cold tests, glutamic acid decarboxylase activity, electrical resistance of seed leachates and tetrazolium reactions. However, they are not as generally applicable and effective as accelerated ageing in predicting the relative storability of seedlots (Delouche & Baskin 1973). Accelerated ageing has also been used as a tool in assessing the relative vigour of seedlots of tree seeds (Elam & Blanche 1989, Blanche *et al.* 1990, Thapliyal & Connor 1997, Singh & Bonner 2001). Downie and Wang (1992) used accelerated ageing to assess the vigour of jack pine, lodgepole pine and white spruce seeds upgraded by the IDS (Incubation, Drying and Separation) technique.

In a previous study, we reported the feasibility of IDS technique to upgrade the quality of *Pinus patula* seedlot by removing dead-filled and empty seeds (Demelash *et al.* 2002). The objective of this study was to evaluate the relative storability of *P. patula* seed lot upgraded by the IDS treatment.

Materials and methods

Seed collection

Seeds of *P. patula* were collected from a plantation in Assela, southeast Ethiopia. The means for annual rainfall and temperature were 1250 mm and 15–20 °C respectively (Anonymous 1988). Seeds were placed in plastic bags and transported to the Swedish University of Agricultural Sciences in Umeå, Sweden. They were stored at 5 °C and 6.8% moisture content in glass bottles until the study was carried out. Two sub-samples were obtained from this seedlot. One of the sub-samples was upgraded with IDS treatment by removing dead-filled and empty seeds.

IDS treatment

To remove empty and dead-filled seeds from *P. patula* seedlot, some seeds were incubated between two moistened germination papers in an incubation cabinet for seven days at 15 °C, 85% relative humidity and 20 μE m^{-2} s^{-1} illumination (fluorescent lamp F 40W/33 RS, cool white light). As this kind of incubation allowed uncontrolled water uptake, any excess water was gently removed with paper towel. After incubation, seeds were spread out on a piece of meshed cloth and dried for three hours in a ventilated drying cabinet set at 20 °C and 40% relative humidity (RH). The IDS conditions used in this study gave an optimal separation of viable and non-viable seeds (Demelash *et al.* 2002). Immediately after drying, seeds were placed in bowls containing water and stirred to differentiate between the floating and sinking portions. The floating and sunken fractions were collected separately after five minutes. The sunken fraction, representing the viable seeds, was further dried down to storage moisture content of 7% using silica gel in a desiccator.

Accelerated ageing procedure

To remove fungal outgrowth after each ageing treatment, the accelerated ageing plastic boxes (22.5 × 19 × 7 cm) with bronze wire mesh seed holders were surface-sterilised with 0.25% mercuric chloride solution and rinsed thoroughly with distilled de-ionised water. A total of 400 sunken seeds from IDS treatment were weighed and placed in a single layer on the surface of the bronze wire mesh seed holder above 250 ml (1 cm deep) de-ionised water. The boxes were then tightly closed and placed in an ageing chamber set at 41 °C and 100% RH for 24, 48, 72, 96, 120, 144, 168, 192 or 216 hours (ISTA 1995). After each time period, seeds were removed from the chamber for germination and leachate conductivity tests. The same numbers of IDS untreated seeds were also aged for comparison and unaged seeds from both IDS-treated and untreated seeds served as controls.

Leachate conductivity test

Leachate conductivity was measured using a single probe conductivity meter. Four replicates of 50 seeds each from each treatment were weighed to two decimal places and placed in 50 ml flasks containing 50 ml de-ionised water. Two flasks with blank samples (50 ml de-ionised water with no seeds) were also prepared and placed together. All flasks were covered with aluminum foil to maintain the temperature and placed at 20 °C. Leachate conductivity was determined after 24 hours of soaking. Several measurements were taken from each sample until a stable value was obtained. The mean of the blank samples was subtracted from each reading and the result was divided by sample weight 24 hours after imbibition to reach at conductivity in $\mu S\ cm^{-1}\ g^{-1}$ of seed (ISTA 1995, Sorensen *et al.* 1997).

Germination test

Germination tests were carried out on Jacobsen's apparatus at 20 ± 1 °C with an illumination of 20 $\mu E\ m^{-2}\ s^{-1}$ (fluorescent lamp F 40 W/33 RS, cool white light) for 21 days (ISTA 1999). Four replicates of 50 seeds each from the IDS-treated and untreated seeds were sown on standard germination paper. Seeds were soaked in 0.25% mercury chloride solution for 10 min and washed thoroughly with distilled de-ionised water prior to sowing to avoid fungal contamination. Germinants were counted when the radicle had reached the size of the seed and had a normal appearance.

Data analysis

Moisture content, percentage germination, mean germination time and leachate conductivity were computed for each trail. Each data set was subjected to ANOVA test and Tukey's test was used to compare differences between periods of accelerated ageing at the 5% level of significance (Zar 1996). Percentage germination was arcsine transformed before analysis to approximate the normality assumption for analysis of variance. A linear regression analysis was also performed

to establish the relationship between leachate conductivity versus germination capacity and mean germination time. Since percentage germination and leachate conductivity did not have a linear relationship, the percentage germination data were log-transformed (log_{10}) prior to fitting the regression model.

Results

Germination capacity and mean germination time

Germination capacity showed a significant difference between IDS-treated and untreated seeds, as well as between periods of accelerated ageing (Table 1). All ageing treatments decreased the germination capacity of the seeds. However, the loss of viability, as determined by germination capacity, was higher in untreated seeds. For example, the germination capacity of untreated seeds dropped to 12% after 72 hours of ageing, while 75.5% germination was obtained in IDS-treated seeds. It was evident that ageing up to 48 hours did not bring a significant reduction in viability in both IDS-treated and untreated seeds compared with the control (unaged seeds).

The mean germination time was also significantly different between the two types of seeds as well as between periods of accelerated aging (Table 2). Mean germination time in both types of seeds increased with increasing periods of accelerated ageing. However, the loss of vigour, as determined by the mean germination time, was significantly higher in untreated seeds. Mean germination time was also initially higher for untreated seeds compared with that of IDS-treated seeds. Accelerated ageing up to 48 hours did not significantly reduce the vigour of either seeds when compared with the unaged controls.

Table 1 Germination capacity (%) of IDS-treated and untreated seeds of *Pinus patula* following accelerated ageing (Mean ± SE)

Accelerated ageing (hours)	IDS treated	Untreated	Overall mean
0	99.25 ± 0.5 a	72.25 ± 4.6 a	85.75 ± 7.8 a
24	97.00 ± 0.6 a	69.50 ± 2.2 a	83.25 ± 7.5 a
48	91.00 ± 1.7 a	68.00 ± 2.2 a	79.50 ± 6.4 a
72	75.50 ± 1.7 b	12.00 ± 0.8 b	43.75 ± 17.0 b
96	72.00 ± 2.9 b	7.00 ± 0.6 bc	39.50 ± 17.5 b
120	71.50 ± 2.6 b	7.00 ± 0.6 bc	39.25 ± 17.3 b
144	70.00 ± 2.5 b	7.00 ± 0.6 bc	38.50 ± 16.9 b
168	55.00 ± 2.4 c	5.00 ± 0.6 bc	30.00 ± 13.5 c
192	41.50 ± 3.3 d	3.50 ± 1.0 bc	22.50 ± 10.4 d
216	13.00 ± 1.3 e	3.00 ± 0.6 c	8.00 ± 2.83 e
Overall mean	68.57 ± 0.6 a	25.43 ± 0.6 b	

Means in the same column followed by the same letter are not significantly different at the 0.05 probability level using Tukey's test.

Table 2 Mean germination time (days) of IDS-treated and untreated seeds of *Pinus patula* following accelerated ageing (Mean ± SE)

Accelerated ageing (hours)	IDS treated	Untreated	Overall mean
0	8.96 ± 0.2 a	13.12 ± 0.2 a	11.04 ± 1.1 a
24	9.29 ± 0.1 a	13.66 ± 0.1 a	11.47 ± 1.2 a
48	9.63 ± 0.0 a	13.67 ± 0.1 a	11.65 ± 1.8 a
72	12.27 ± 0.3 b	15.29 ± 0.3 ab	13.78 ± 0.8 b
96	13.33 ± 0.3 bc	16.84 ± 0.6 bc	15.08 ± 1.0 c
120	13.35 ± 0.1 bc	17.83 ± 0.3 cd	15.59 ± 1.2 cd
144	13.44 ± 0.2 c	18.13 ± 0.8 cde	15.78 ± 1.4cd
168	13.62 ± 0.5 cd	19.17 ± 0.6 de	16.39 ± 1.6 de
192	14.55 ± 0.1 d	19.88 ± 0.7 de	17.21 ± 1.5 e
216	19.12 ± 0.2 e	20.25 ± 0.5 e	19.68 ± 0.5 f
Overall mean	12.75 ± 0.1 a	16.70 ± 0.1 b	

Means in the same column followed by the same letter are not significantly different at the 0.05 probability level using Tukey's test.

Moisture content and leachate conductivity

Moisture content (Figure 1) and leachate conductivity values (Table 3) increased as ageing progressed from 24 to 216 hours in both seeds. Leachate conductivity values for untreated seeds were significantly higher than those of IDS-treated seeds. The result indicated that germination capacity dropped below 50% when leachate conductivity exceeded 50 μS cm^{-1} g^{-1} in both types of seeds, although the decline in germination was faster in untreated seeds. Moisture content did not differ significantly between the two types of seeds, but it was significantly different between periods of accelerated ageing (Figure 1).

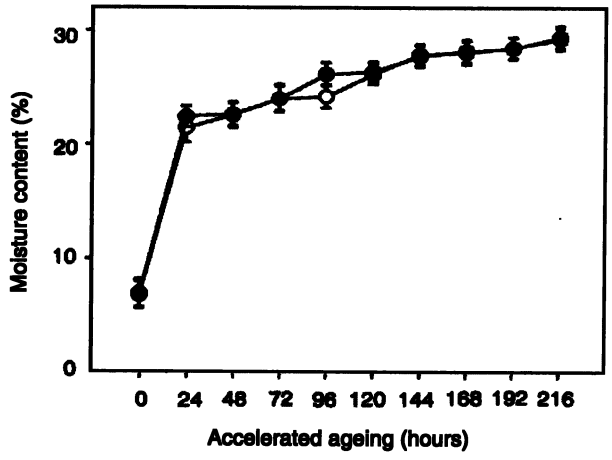

Figure 1 Moisture contents of IDS-treated (filled circles) and untreated seeds (hollow circles) of *Pinus patula* following accelerated ageing

Table 3 Leachate conductivity values (μS cm^{-1} g^{-1}) of IDS-treated and untreated seeds of *Pinus patula* following accelerated ageing (Mean ± SE)

Accelerated ageing (hours)	IDS treated	Untreated	Overall mean
0	10.19 ± 0.1 a	36.16 ± 1.7 a	23.18 ± 7.0 a
24	19.24 ± 0.5 ab	36.78 ± 2.8 a	28.01 ± 5.0 a
48	21.58 ± 1.1 bc	37.03 ± 2.1 a	29.30 ± 4.4 a
72	30.85 ± 1.2 cd	60.32 ± 1.8 ab	45.59 ± 7.9 b
96	37.68 ± 2.4 de	86.10 ± 4.3 bc	61.89 ± 13.3 c
120	38.27 ± 2.1 de	94.13 ± 4.6 cd	66.20 ± 15.3 d
144	40.48 ± 2.9 de	118.74 ± 6.0 de	79.61 ± 21.4 de
168	48.15 ± 2.5 ef	120.66 ± 6.3 e	84.40 ± 19.9 e
192	54.36 ± 2.7 f	150.85 ± 12.7 f	102.60 ± 27.2 f
216	152.51 ± 4.3 g	160.44 ± 2.6 f	156.47 ± 3.9 g
Overall mean	45.33 ± 1.3 a	90.12 ± 1.3 b	

Means in the same column followed by the same letter are not significantly different at the 0.05 probability level using Tukey's test.

The relationship between leachate conductivity and germination

There was a strong negative correlation (Figure 2) between leachate conductivity and germination capacity in both IDS-treated (r = -0.986) and untreated seeds (r = -0.930). Likewise, the mean germination time was strongly positively correlated (Figure 3) with leachate conductivity for untreated (r = 0.984) and IDS-treated seeds (r = 0.914).

The fitted linear regression equations were

(1) Log germination percentage = 2.064 – 0.011 *LC*; r^2 = 0.865 for untreated seeds,

(2) Log germination percentage = 2.077 – 0.0065 *LC*; r^2 = 0.972 for IDS-treated seeds,

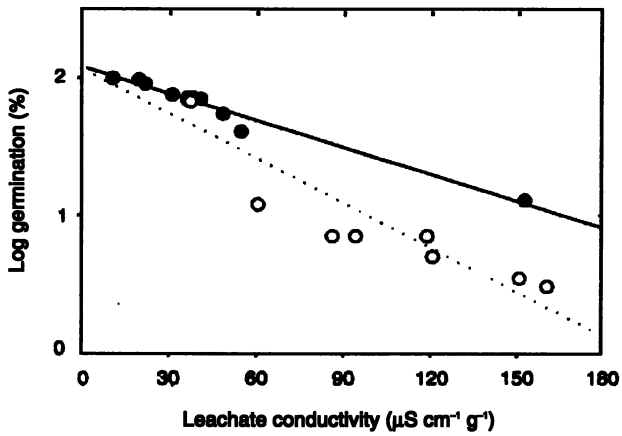

Figure 2 Relationship between leachate conductivity and germination capacity of IDS-treated (filled circles) and untreated (hollow circles) *Pinus patula* seeds

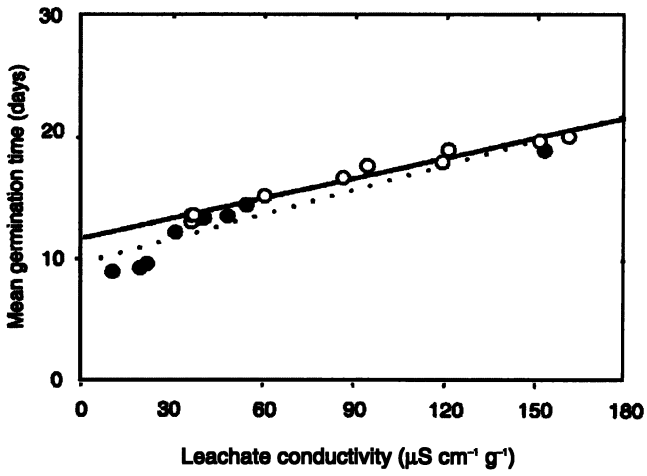

Figure 3 Relationship between leachate conductivity and mean germination time of IDS-treated (filled circles) and untreated (hollow circles) *Pinus patula* seeds

(3) Mean germination time = 11.71 + 0.056 *LC*; r^2 = 0.968 for untreated seeds,
(4) Mean germination time = 9.63 + 0.069 *LC*; r^2 = 0.836 for IDS-treated seeds,

where *LC* = leachate conductivity. The r^2 values of the fitted models indicated that the models explained the linear relationship between leachate conductivity versus germination capacity and mean germination time very well.

Discussion

All accelerated ageing treatments decreased the germination capacity and increased the mean germination time of IDS-treated and untreated seeds (Tables 1 and 2). The first process during seed deterioration due to ageing is excessive leakage of cytoplasmic components as a result of loss of membrane integrity (Basavarajappa *et al.* 1991, Bewley & Black 1994, Smith & Berjak 1995). This is clearly evident from the increased leachate conductivity values with ageing in this study (Table 3). Kalpana and Madhava (1995) reported an increase in initial water uptake and leakage of cellular compounds and a marked decline in oxygen consumption in pigeonpea cultivars with increasing periods of ageing. The increase in moisture content and amino nitrogen with gradual decrease in starch content during accelerated ageing in *Quercus nigra* seed was also reported by Blanche *et al.* (1990). Similarly, Kataki *et al.* (1997) reported that the reduction in sucrose and raffinose correlated with the decline in seed germinability in maize and rice. As reviewed by Walters (1998), numerous authors have reported decline of activities of several enzymes. Recently, Shen and Odén (1999, 2000) have shown a decline in fumarase activity, a key respiratory enzyme in the TCA cycle, with ageing in Scots pine seeds. These overall metabolic events during ageing eventually deplete food reserve and subsequently seed vigour declines. Furthermore, Madhava and Kalpana (1994) suggested that the accumulation of reducing sugar

in pigeonpea seeds during accelerated ageing played a critical role in reducing seed viability. It has been shown that an accumulation of reducing sugars could lead to the occurrence of Amadori and Maillard reactions that may cause protein inactivation and nucleic acid damage and hence trigger seed deterioration (Wettlaufer & Leopold 1991). The Amadori reaction involves a simple non-enzymatic glycation of reducing sugars on amino groups within proteins to form fructyosyl derivatives or glycated proteins while the Maillard reaction involves subsequent complex interactions between the glycated Amadori products to form complex brown-coloured compounds. On top of this, changes in seed coat surface elemental composition with ageing have been observed in *P. taeda* and *P. roxbughii* seeds (Vanangamudi *et al.* 1998) that may have a bearing on membrane integrity.

The decline in germination capacity and an increase in mean germination time with accelerated ageing observed in the present study accord with previous studies on tree seeds (Elam & Blanche 1989, Blanche *et al.* 1990, Thapliyal & Connor 1997, Rehman *et al.* 1999, Vanangamudi *et al.* 2000, Masilamani & Dharmalingam 2001, Singh & Bonner 2001). Downie and Wang (1992) also showed that accelerated ageing reduced the mean germinability of jack pine, lodgepole pine and white spruce seedlots and seed fractions separated by IDS technique although the mean index of ageing was not significantly different between the control and the bottom fraction (live seeds).

Seed moisture content increased with periods of accelerated ageing (Figure 1). The gradual reduction in germination with increasing moisture content following ageing suggests that moisture content is probably one of the most important factors affecting seed viability. The increased moisture content could be sufficient to induce metabolic activities but not protrusion of the radicle. As a result, seed deterioration occurs because of energy expenditure (utilisation of food reserves) or microbial attack (Leopold & Vertucci 1989). Furthermore, the increased activities of phospholipases destroy the membrane structure of the seed (Basavarajappa *et al.* 1991, Pukacka 1993, Salama & Pearce 1993, Copeland & McDonald 2001). Other workers have also reported an increase in moisture content with ageing (Elam & Blanche 1989, Blanche *et al.* 1990, Singh & Bonner 2001).

Leachate conductivity was strongly negatively correlated with germination capacity (Figure 2) and positively correlated with mean germination time (Figure 3) for both types of seeds. Thornton *et al.* (1990) have shown a significant correlation between leachate conductivity and germination of *Brassica* seeds, Thapliyal and Connor (1997) for *Dalbergia sissoo* seeds, and Elam and Blanche (1989) for slash pine, longleaf pine, loblolly pine, water oak and pecan. Our study, thus, supports the utility of leachate conductivity as a routine supplement or a quick alternative to assess the germination capacity (viability) and the mean germination time (vigour) of *P. patula* seeds as suggested by other authors (Bonner 1991b, Sorensen *et al.* 1997, Singh & Bonner 2001).

The study revealed that IDS-treated seeds could withstand stress established during accelerated ageing better than their untreated counterparts. This could be attributed to the invigoration effect induced during IDS treatment. Actually, the IDS technique was initially developed to upgrade the quality of conifer seedlots by removing empty and dead-filled seeds (Simak 1981, 1984). However, Bergsten

(1987, 1988) modified the incubation step to include an invigoration step in Goretex membrane tubes for low vigour seeds. Although the invigoration step used by Bergsten (30% initial moisture content and seven days of incubation at 15 °C) was not exactly adopted in our study, we thought that the IDS condition used in our study (free access to water for seven days at 15 °C) might have enhanced the vigour of *P. patula* seeds. It is well known that high vigour seeds tolerate the stress induced during accelerated ageing better than low vigour seeds (Hartmann *et al.* 1997) and seedlots that maintain germination well during accelerated ageing will have a high storage potential (Delouche & Baskin 1973).

Conclusions

IDS-treated seeds maintained relatively higher germination following periods of accelerated ageing than untreated seeds. It can, therefore, be concluded that *P. patula* seedlots upgraded by IDS treatment would have higher storability than the untreated seeds. This enables tree seed companies to continually supply their customers with high quality seeds. There was a strong negative correlation between leachate conductivity versus germination capacity and a positive correlation between leachate conductivity and mean germination time. Hence, we suggest the leachate conductivity test as a routine supplemental test or as a quick alternative test to predict the viability and vigour of *P. patula* seedlots.

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