Seed viability constants for *Eucalyptus grandis*

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Abstract – This work aimed to analyse *Eucalyptus grandis* W.Hill ex Maiden seed behaviour, under controlled deterioration, and to estimate viability equation constants for the species. Seeds were harvested in the growing season of 1999, and the moisture contents were adjusted from 11.3% to a range between 1.2 and 18.1% at 25ºC. The subsamples were sealed into laminate aluminium-foil packets, for storage in incubators at 40, 50 and 65±0.5ºC. The seeds presented orthodox performance, in which the constants for predicting seed longevity of *E. grandis* were K_E = 9.661, C_W = 6.467, C_H = 0.03498 and C_Q = 0.0002330. The usual and inverse relationship between water content and seed longevity was also observed. The lowest moisture content limit for application of the viability equation at 65ºC was 4.9%, estimated under hygroscopic equilibrium with 23% of relative humidity in the storage environment.

Index terms: seed deterioration, storage, seed longevity.

Constantes de viabilidade para sementes de *Eucalyptus grandis*

Resumo – Este trabalho teve como objetivos verificar o desempenho de sementes de *Eucalyptus grandis* W.Hill ex Maiden, após a deterioração em condições controladas, e obter as constantes da equação de viabilidade. As sementes foram colhidas na safra de 1999, e a umidade foi ajustada de 11,3% para valores entre 1,2 e 18,1% a 25ºC As subamostras foram acondicionadas em embalagens de alumínio termossoldadas, armazenadas a 40, 50 e 65±0,5ºC. As sementes apresentaram um desempenho ortodoxo em relação ao armazenamento. As constantes para a predição da longevidade foram K_E = 9,661, C_W = 6,467, C_H = 0,03498 e C_Q = 0,0002330. Foi observada a relação inversa entre teor de água e longevidade. O limite inferior de grau de umidade, calculado para aplicação da equação a 65ºC, foi de 4,9%, estimativa obtida sob equilíbrio higroscópico com umidade relativa de 23% no ambiente de armazenamento.

Termos para indexação: deterioração de sementes, armazenamento, longevidade de sementes.

Introduction

Brazil occupies a remarkable position in international forestry technology, mainly as for *Eucalyptus* spp., presenting a great concentration of paper, cellulose and furniture production corporations. Forestation activity could provide good economical return, due to high productivity and economical value of the products, despite a reforestation reduction was observed during the last decade in Brazil, mainly in São Paulo State (Fundação Florestal, 2003).

The maintenance of a germplasm bank is relevant for the conservation of forest species; however, a specific methodology for seed storage is not yet available. The establishment of this methodology is the chief aim of seed researchers, technologists and producers (Usberti & Gomes, 1998).

The period in which seed viability could be maintained gives relevance to forest species storage researches, which present an uneven seed production, abundant in certain years, and scarce in others. *E. grandis* plays an important role in the availability of forest raw material for short and long fibre cellulose production. The studies with forest species of commercial use, under controlled storage conditions, could contribute for seed viability preservation (Silva, 2001).

Bonner (1990) proposed a classification of forest species seed behaviour in storage, as follows: true orthodox (tolerated drying is below 10% moisture content and storage at subzero temperatures); suborthodox (lower storability than orthodox seeds); recalcitrant temperate (sensitive to drying under low moisture content levels, and could be long-term stored around freezing); recalcitrant tropical (could be stored in high relative humidity , with higher sensitivity to low temperatures and desiccation).

The viability equation of Ellis & Roberts (1980) could predict changes in viability on orthodox seeds, during storage at different moisture content and temperatures. However, viability constants are specific for each species and, therefore, must be determined for species of economic, ecological and scientific interest.

This research aimed to determine the performance of *E. grandis* seeds under controlled deterioration (constant temperatures and moisture content), and to estimate viability constants for the species.

Material and Methods

The experiment was carried out in the Laboratory of Post-Harvest Technology at Faculdade de Engenharia Agrícola, Universidade de Campinas, Brazil, from 2000 to 2003, using a seed sample of *E. grandis* harvested in the growing season of 1999.

A subsample of 5 g was analysed to detect the presence of pure seeds, other seeds and inert material (International Seed Testing Association, 2004), and the fruit and seed relation were determined according to the amount of capsules necessary to achieve 1 kg of mixed seeds.

One thousand seed weight test was performed using eight replicates containing 100 seeds (International Seed Testing Association, 2004). Previously, biological components used in the curve fitting, as the lipid content, were determined in a work sample of 2 g of seeds, using a paper cartridge Whatman (80 mm length x 33 mm internal diameter), and the extraction was performed with ether of petroleum, in a Soxhlet apparatus during 5 hours, to achieve the amount of lipids determined gravimetrically (Association of Official Analytical Chemists, 1995).

Seed moisture contents observed (1.2; 2.9; 4.7; 7.5; 11.3; 14.4; 16.3 and 18.1%), adjusted at 25ºC, were obtained from an initial value of 11.3%, either by rehydration, on a water column of 3 cm in a plastic box, or by dehydration with silica gel. Seed sample for each moisture content was sealed in an aluminium foil packet, and stored during 5 days, at 20°C, to achieve hygroscopic equilibrium. The combinations between temperature and seed moisture content were: 40ºC, with 7.5, 11.3, 14.4, 16.3, and 18.1%; 50ºC, with 1.2, 2.9, 4.7, 7.5, 11.3, 14.4, 16.3, and 18.1%; 65ºC, with 1.2, 2.9, 4.7, 7.5, 11.3, 14.4, 16.3, and 18.1%.

The water activity (A_w) was calculated at 25±0.3ºC, using three replicates for each moisture content obtained after rehydration or dehydration processes, in a hygrometer using the technique of the chilled-mirror dew point (±0.01 A_w). When two A_w values were less than 0.001 apart, the measurement was finished and the water activity displayed. Seed moisture content, in a fresh weight basis, was determined using three replicates of 5 g for each moisture content, under temperature of 130±2ºC, for 2 hours (International Seed Testing Association, 2004).

Fifteen subsamples for each combination between moisture content and temperature were sealed in laminate aluminium foil packets (0.15 g, around 250 seeds), and stored into incubators at 40, 50 and 65±0.5°C. The temperatures were chosen in order to get the complete survival curves in relatively short periods of time. Seed subsamples were taken at intervals and tested for germination, until complete survival curves were obtained (Ellis & Roberts, 1980).

Germination tests were performed on 4 replicates of 0.15 g into a plastic box (11x11x4 cm), on filter paper sheets previously moistened with 6 mL of distilled water. Seed samples germinated into incubators with alternate regime of 20–30ºC, and normal seedling counts were performed at 5 and 14 days. The criterion for seed germination was the protrusion of radicle (Dickie & Smith, 1995). Seed samples with moisture content lower than 7.5% were rehydrated at 25°C, during 48 hours before germination test, in order to avoid imbibition injury during rehydration (Ellis et al., 1988).

Germination data in percentages after storage under each moisture content and temperature combination were scattered in X-Y graphics, and regression lines were fitted to probit values through Y-axis scale transformation.

The residual deviances for seed survival curves at different temperatures were analysed to give the best fit to the viability equation. Statistical analyses were performed using Tukey test at $p<0.05$ for comparison among germination means, with or without previous rehydration

The effect of moisture content and temperature on seed longevity, represented by the sigma value, i.e., the frequency of distribution of seed deaths in time or, in a practical way, the number of days required for the percentage of seed germination to be reduced by one probit, was achieved using the multiple regression analysis within the generalized linear model (Baker & Nelder, 1978; Hay et al., 2003).

Results and Discussion

Physical purity of *E. grandis* seed sample revealed the presence of two different kinds of materials: one was small, light, with filiform format and red colour (sterile ovules), and the other was large, round format and black colour. The percentage of pure seeds detected was 19%. The1000-seed weight for the species was 0.226 g. Taking into account this value and the physical purity percentage obtained, it was achieved a value of 841,643 pure seeds per kg, close to the value of 700,000 pure seeds per kg recorded by Vieira & Diniz (1995).

Seeds lost water quickly in the desorption process beginning (Table 1), and seed moisture content presented inverse relationship with desorption period. Hall (1980) reported that during desorption an increase in the temperature occurs, due to the heat transfer not compensated by the transfer of the seed mass.

After a drying period of two days, seed sample presented moisture content of 6.8% (Figure 1), probably due to lipid content detected in the seeds (24.2%). The desorption process over silica gel presented positive results, without damages in the seeds, even at the lowest moisture content levels achieved, showing similarity with the results obtained by Hu et al. (1998).

No statistical difference was observed for germination percentages among moisture content levels, as well as for seeds with or without rehydration (Table 2). High germination percentage results were obtained at 7.5 and 11.3% moisture content without rehydration, and the lowest value was detected at 1.2% moisture content without rehydration, confirming the need for rehydration before germination test of seeds with moisture content lower that 6%.

E. grandis seeds reached moisture content values from 1.2 to 19.1%, with equilibrium of relative humidity (RH) ranging from 8 to 93% (Table 3). Figure 2 displays sorption and desorption isotherms for experimental and predicted values through Langmuir model (Langmuir, 1918), depending on moisture content obtained above or below initial value (11.3%). The isotherms reveal three areas, corresponding to the different categories of binding water occurring in the tissues; at low moisture content, the water

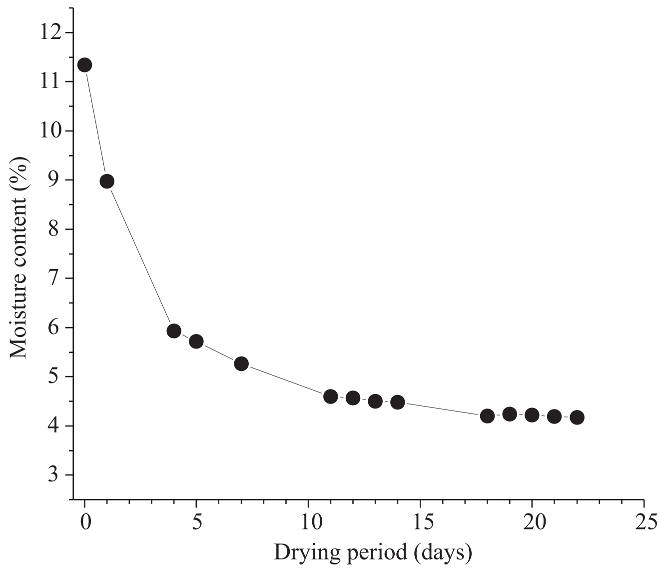

Figure 1. Seed drying rate of *Eucalyptus grandis* over silica gel at 25°C.

Table 1. Period of time for dehydration or rehydration necessary to achieve the desired moisture content levels (%, fresh weight basis) for *Eucalyptus grandis* seeds, from the initial value of 11.3%.

Moisture content (%)	Dehydration (days)	Rehydration (days)
1.2	22.0	-
2.9	4.0	-
4.7	1.0	-
7.5	0.4	-
14.4	-	1.0
16.3	-	1.8
18.1	-	7.7

Table 2. Percentages of seed germination of *Eucalyptus grandis* under different moisture contents, with or without previous rehydration(1).

Moisture content (%)	With rehydration (%)	Without rehydration (%)
11.3	82.5aA	87.5aA
7.5	70.5aA	84.5aA
4.7	79.0aA	78.0aA
2.9	80.0aA	68.0aA
1.2	77.0aA	68.0aA

(1)Estimates followed by the same lower case letters in the column, or upper case letters in the line, don´t differ among themselves by Tukey test at 5% probability.

in the seeds is maintained by very strong binding sites, while for intermediate and high moisture content, the water is maintained by weak binding sites and by the multimolecular water (Vertucci & Leopold, 1987).

Seed survival curves obtained at 40, 50 and 65°C are depicted in Figure 3, showing the effects of moisture content and temperature on seed storability.

The normal distribution in seed longevity and the survival curves obtained show a negative cumulative sigmoidal format, confirming the observation made by Ellis (1984). When moisture content (ranging from 1.2 to 18.1%) and storage temperature were reduced, predictable increasing in seed longevity was observed.

Seeds stored at 40ºC, with 7.5% moisture content, required 90 days for the germination drop by one probit (sigma), while seeds with 14.4% moisture content had germination lower than 5%, after ten days of storage. At 50°C and 4.7, 7.5 and 14.4% moisture content, sigma values estimated were 55, 40 and only 0.62 days, respectively; seed germination was lower than 8% after 0.184 days at 8.1% moisture content. For seeds stored at 65ºC, with 1.2, 7.5, 11.3 and 16.3% moisture content, sigma values were 40, 1, 0.38 and 0.093 days, respectively; moreover, seed germination value was lower than 5%, after 96 days of storage at 2.9% moisture content.

Increases in temperature and moisture content resulted in seed longevity reduction, more pronounced at the highest moisture contents (16.3 and 18.1% at 50°C). Similar results were obtained in groundnut (Usberti & Gomes, 1998), and in *Dalbergia nigra* and *Dimorphandra mollis* (Chaves & Usberti, 2004).

Table 4 displays seed moisture content and sigma values for *E. grandis* stored at 40, 50 and 65°C. Seeds with 18.1% moisture content, stored at 40 and 50°C, required 0.736 and 0.184 day for the germination drop by one probit, while with 17% moisture content, at 40 and 50°C, those values were 0.029 and 0.007 day, respectively.

According to Ellis & Roberts (1980) and Ellis et al. (1982, 1988, 1989), there is a quadratic relationship between seed longevity and temperature, as well as a negative logarithmic relationship occurs between seed longevity and moisture content in several species. Moreover, Ellis et al. (1990) reported the occurrence of a similar relative effect of seed water potential on eight vegetable species and, probably, this phenomenon occurs similarly in other orthodox species.

Tompsett (1986) verified that moisture content increase in seeds stored at constant temperature causes a decrease in longevity, in all temperatures, and it could be predicted through the viability equation of Ellis & Roberts (1980). The initial seed quality (Ki value) for *E. grandis* was estimated in 1.351, through 21 combinations of storage temperatures and moisture content.

The best statistical adjustment for *E. grandis* viability equation was obtained after removing the data of 1.2, 2.9 and 4.7% moisture content at 65ºC, and 4.7% moisture content at 50ºC.

The constant K ($K = K_E - C_H t - C_Q t^2$) gives a simplified estimate of seed viability when only one storage temperature is considered. The constants obtained for the species define the equation at 40, 50

Table 3. Estimates of water activity (A_w) for *Eucalyptus grandis* seeds.

Moisture content (%)	Aw
1.7	0.087
3.3	0.096
4.9	0.228
8.0	0.397
11.3	0.654
14.8	0.806
15.9	0.858
18.5	0.936

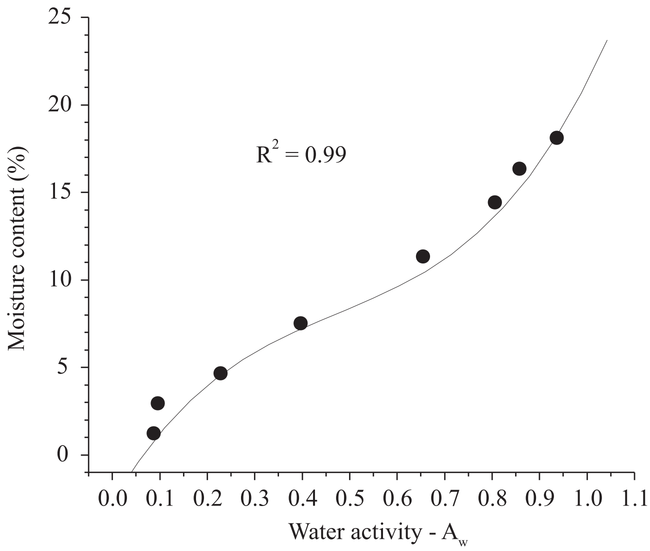

Figure 2. Sorption and desorption isotherms for seeds of *Eucalyptus grandis*.

and 65°C, as follows: K = 8.084, 5.745, 2.486, and C_W = 6.641, 5.103, 2.777, respectively.

The lower moisture content limit for application of the viability equation at 65ºC supplies a practical orientation for seed drying before storage, and means the discontinuity of the logarithmic relationship between seed moisture content (fresh weight basis) and sigma for the species. This value calculated for *E. grandis* was 4.9% moisture content, in equilibrium with 23% RH.

This moisture content limit is variable and was evaluated in 23 species by Roberts (1991). For instance, this value was 2% moisture content for peanut and sunflower (Ellis et al., 1988, 1990), respectively, and 6.2% for pea seeds (Ellis et al., 1989).

The values calculated for the viability constants K_E, C_W, C_H and C_Q obtained for *E. grandis* were 9.661, 6.467, 0.03498 and 0.0002330, respectively. Those values are closer to the obtained for barley seeds by Ellis & Roberts (1980), as follows: K_E = 9.983, Cw = 5.896, C_H = 0.040 and C_Q = 0.000428. Tompsett (1989) reported that those constants could predict the seed longevity safely, in any homogeneous seed lot under variable storage conditions.

The constant C_W varies among the species and was estimated around 6 for cereal seeds, while for oleaginous species it ranged from 3.5 to 4, so indicating the need of the oleaginous seeds to be dried at lower moisture content levels than cereal ones (Ellis, 1984).

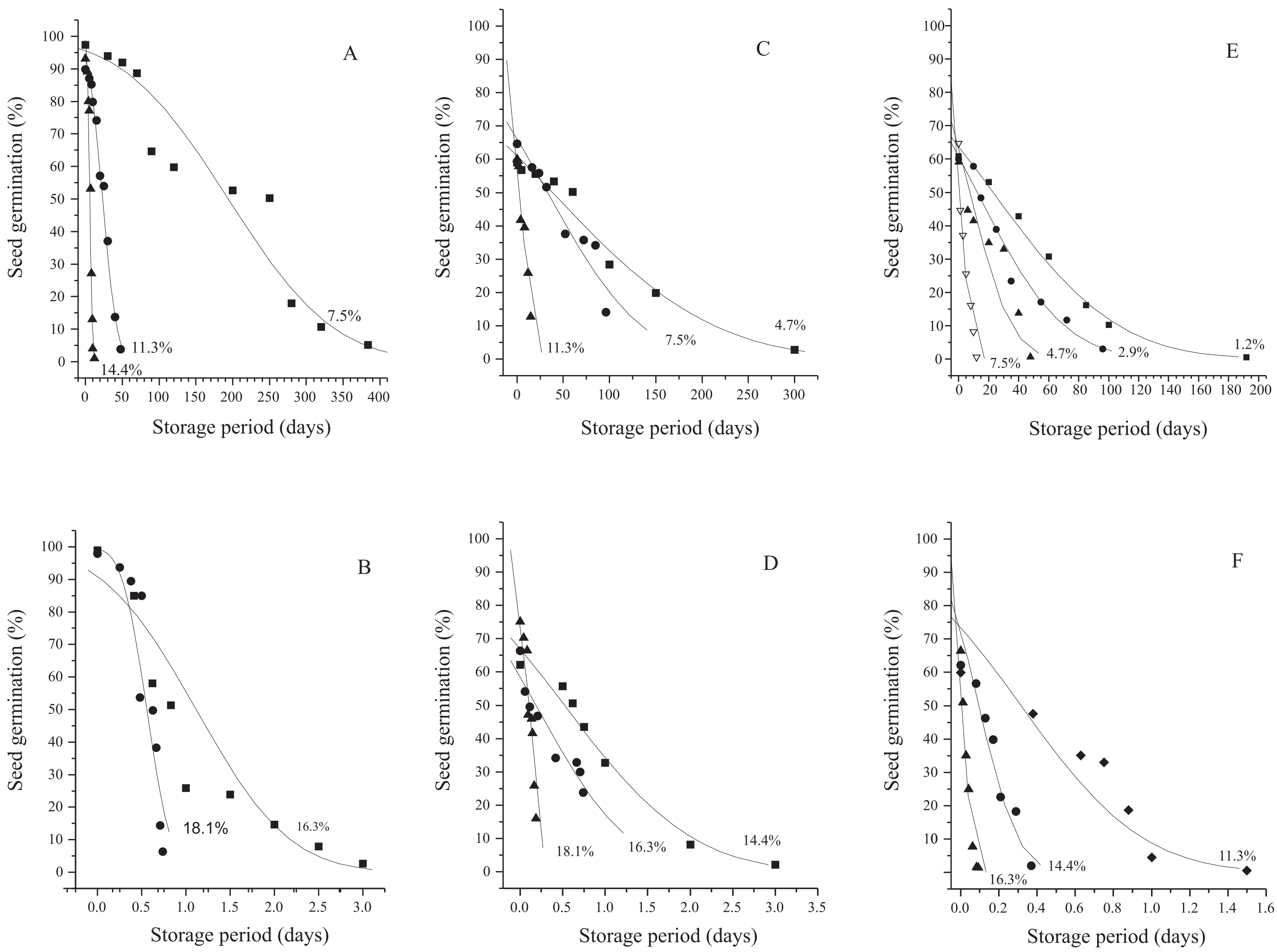

Figure 3. Probit survival curves for *Eucalyptus grandis* seeds, stored at 40°C, with moisture contents of 7.5, 11.3, 14.4% (A), and 16.3, 18.1% (B); at 50°C, with moisture contents of 4.7, 7.5, 11.3% (C), and 14.4, 16.3, 18.1% (D); at 65°C, with moisture contents of 1.2, 2.9, 4.7, 7.5% (E), and 11.3, 14.4, 16.3% (F). Symbols and straight lines mean germination percentages and survival curves, respectively.

Dickie et al. (1990) observed that the viability equation could be applied to storage temperatures ranging from -13 to 90°C, and detected a small and negative association between C_W and seed lipid level among different species.

The viability equation, obtained in this work, for *Eucalyptus grandis* is:

$v = Ki\text{-}p/10^{9{,}661-6.467\log m-0.03498t-0.0002330t^2}$

Several economically valuable forest species with orthodox seeds, like *Abies*, *Picea* and *Pinus*, and some tropical species including *Acacia* and *Eucalyptus*, could be long-term stored at low temperatures, since their seed moisture content were reduced to level lower than 10% (Bonner, 1990).

Figure 4 displays the logarithmic relationship between seed moisture content and sigma at 40, 50 and 65°C, through parallel straight lines, specific for each storage temperature applied.

Chaves & Usberti (2004) achieved two different groups of viability constants to predict seed longevity in *Dalbergia nigra* and *Dimorphandra mollis*, as follows: K_E = 5.199 and 6.282, C_W = 4.524 and 3.838, C_H = 0.08175 and 0.05405, C_Q = 0.001641 and 0.001316, respectively. For the two species, an inverse relationship was observed between moisture content and seed longevity.

According to Bonner (1999), the viability equations for the forest species *Pinus taeda*, *P. elliottii*, *Liquidambar styraciflua* and *Platanus occidentalis* were K_E = 1.8486, 5.5557, 5.6611 and 4.7477; C_W = -2.2449, 1.3787, 2.1515 and 1.3413; C_H = 0.0514, 0.0398, 0.0280 and 0.0392; C_Q = -0.00014, 0.0008, 0.0009 and 0.0007, respectively. There was no relationship among seed lipid level and the moisture content constant (C_W) for those species.

The viability constants were also reported for groundnut seeds, as follows: K_E = 6.177, C_W = 3.426, C_H = 0.0304 and C_Q = 0.000453. The calculated lowest moisture content limit for application of the equation at 65°C was 2.4% (Usberti & Gomes, 1998).

Medeiros et al. (1998), using the estimated values of K_E = 7.5498 and C_W = 3.76, and adopting C_H = 0.0329 and C_Q = 0.000478 as universal constants, reported that the species *Astronium urundeuva* presented an orthodox behaviour during storage, and taking into account the storage conditions of -20°C and seed moisture content in equilibrium with 15°C and 15% RH, the time predicted for seed viability drop by one probit was calculated in 1,167 years.

At storage conditions of 4°C (domestic refrigerator) and seed moisture content of 7.5% (in equilibrium with 45% RH), obtained in a few days in silica gel, it is expected that *E. grandis* will require 19.9 years for seed viability drops by one probit.

Table 4. Moisture content (%) and sigma (number of days for the percentage of seed germination to be reduced by one probit) for *Eucalyptus grandis* stored at 40, 50 and 65ºC, after constraining the survival curves to same origin point.

Moisture content	Sigma (days)		
	40°C	50°C	65°C
1.2	-	-	43.20
2.9	-	-	27.80
4.7	-	97.3	14.50
7.5	137.6	40.8	3.60
11.3	17.3	5.0	0.40
14.4	4.2	0.8	0.10
16.3	0.8	0.3	0.03
18.1	0.4	0.1	-

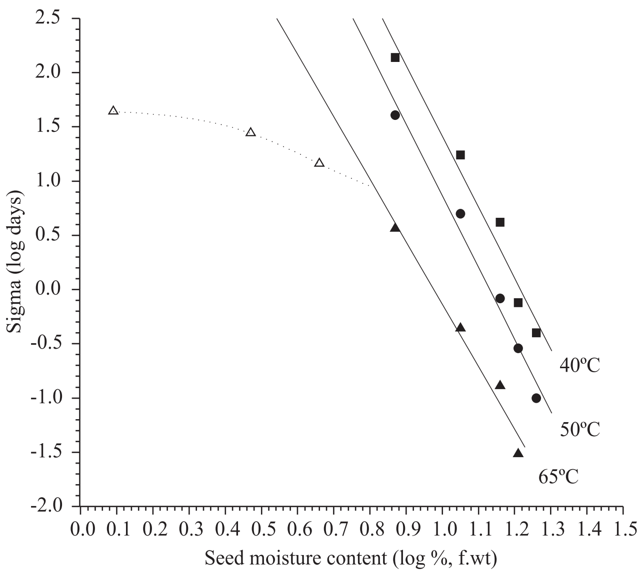

Figure 4. Logarithm relationship between moisture contents (fresh weight basis) and the standard deviation for frequency distribution of deaths in time (sigma) for *Eucalyptus grandis* seeds stored at 40, 50 and 65°C. Solid lines stand for slopes which are constrained to one common value. Open symbols mean moisture content values removed to get best equation adjustment.

Conclusions

1. *Eucalyptus grandis* presents orthodox seed behaviour in storage.

2. There is an inverse relationship between moisture and seed longevity.

3. A set of viability constants to predict *Eucalyptus grandis* seed longevity is: $K_E = 9.661$, $C_W = 6.467$, $C_H = 0.03498$, $C_Q = 0.0002330$.

4. The lowest seed moisture content limit for viability equation application at 65ºC is 4.9%.

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