

COMPARISONS BETWEEN TWO ECONOMICALLY VALUABLE FOREST SPECIES (*Eucalyptus grandis* AND *Pinus taeda*) IN RELATION TO SEED BEHAVIOUR UNDER CONTROLLED DETERIORATION¹

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ABSTRACT – The objectives of this work were to analyze seed behaviour under controlled deterioration and estimate viability equations for forest species *Eucalyptus grandis* and *Pinus taeda*. Desired moisture content levels were achieved from initial values after either rehydration over water or drying over silica gel, both at 25 °C. Seed sub samples with 8 moisture contents each for *E. grandis* (1.2 to 18.1%, initial value of 11.3%) and *P. taeda* (1.5 to 19.5%, initial value of 12.9%) were sealed in laminate aluminium-foil packets and stored in incubators maintained at 40, 50 and 65 °C. The seeds from these species exhibited true orthodox and sub-orthodox storage behaviour, respectively, however *E. grandis* showed higher seed storability, probably due to a different seed chemical composition. Lowest moisture content limits estimated for application of the viability equations at 65 °C were 4.9 and 4.1 mc for *E. grandis* and *P. taeda*, on equilibrium with ±20% RH. The viability equation estimated quantified the response of seed longevity to storage environment well with $K_E = 9.661$ and 8.838 ; $C_w = 6.467$ and 5.981 ; $C_H = 0.03498$ and 0.10340 ; $C_Q = 0.0002330$ and 0.0005476 , for *E. grandis* and *P. taeda*, respectively. .

Keywords: Forest species, Seed storability and Viability equation.

COMPARAÇÕES ENTRE DUAS ESPÉCIES FLORESTAIS DE VALOR ECONÔMICO (*Eucalyptus grandis* AND *Pinus taeda*) COM RELAÇÃO AO COMPORTAMENTO DAS SEMENTES SOB DETERIORAÇÃO CONTROLADA

RESUMO – Os objetivos deste trabalho foram analisar o comportamento de sementes sob deterioração controlada e estimar as equações de viabilidade das espécies florestais *Eucalyptus grandis* e *Pinus taeda*. Os graus de umidade desejados foram obtidos a partir dos valores iniciais após a reidratação sobre água ou secagem sobre sílica-gel, ambos a 25 °C. Subamostras de sementes com 8 C graus de umidade cada de **E. grandis** (1,2 a 18,1%, valor inicial de 11,3%) e de **P. taeda** (1,5 a 19,5%, valor inicial de 12,9%) foram colocadas em envelopes laminados de alumínio e mantidas em incubadoras a 40, 50 e 65 °C. O comportamento das espécies durante o armazenamento foi ortodoxo e subortodoxo, respectivamente. Entretanto, o potencial de armazenamento de sementes de **E. grandis** foi maior, talvez devido à diferente composição química das sementes. Os limites inferiores de graus de umidade para aplicação da equação de viabilidade a 65 °C foram 4,9 e 4,1% em **E. grandis** e **P. taeda**, em equilíbrio com ±20% UR. As equações de viabilidade estimadas quantificam bem a resposta da longevidade das sementes ao ambiente de armazenamento, como se segue: $K_E = 9,661$ e $8,838$; $C_w = 6,467$ e $5,981$; $C_H = 0,03498$ e $0,10340$; $C_Q = 0,0002330$ e $0,0005476$, em **E. grandis** e **P. taeda**, respectivamente.

Palavras-chave: Espécies florestais, Armazenabilidade de sementes e Equação de viabilidade.

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1. INTRODUCTION

Eucalyptus spp. and *Pinus* spp. are the main sources for supplying raw material for the production of short and long fiber cellulose and mechanical processing and have been established around 3,800,000 ha of Brazilian reforested areas.

Seed storability is affected mainly by moisture content (mc) and storage temperature (T). According to Harrington (1963), mc is the most important factor in seed longevity and the viability period could be doubled after reductions of either 1% in mc or 5.6°C in T.

Roberts (1973) classified the seeds in storage as orthodox, showing a medium life around 3-15 years, with seed longevity inversely proportional to T and mc whereas recalcitrant seeds have short viability and more difficult conservation. The orthodox seed behavior can be investigated thereby reducing both moisture content and temperature, and their longevity can be described in a quantifiable and predictable way.

Bonner (1990) proposed a specific classification for forest species: true orthodox, which tolerates desiccation at low T and maintains seed viability for long periods in dry conditions (*Eucalyptus*, *Pinus* and *Acacia*); sub-orthodox, which could be stored in the same conditions above for the maximum of 6 years, presenting high lipid content, small size and fine seed coats; recalcitrant temperate, which are sensitive to desiccation but could be stored at T around freezing (*Quercus* spp.) and recalcitrant tropical, presenting big seeds and being sensitive to desiccation and freezing (coconut, cocoa and mango).

Several equations have been proposed to relate storage conditions with seed viability, aiming at predicting seed survival as well as seed sensibility to mc and T. The viability equation presented by Ellis and Roberts (1980) ($v = K_i p/10^{K_E C_W \log m C_H T C_Q^2}$) allows estimates of any seed lot viability during storage at different T and mc and includes five constants, as follows: one quantifying the initial seed lot quality (K_i), one for each species (K_E), one indicating the logarithmic response of seed longevity to mc (C_W) and two constants for linear and quadratic T (C_H and C_Q , respectively).

The main objectives of this work were (1) analyzing *E. grandis* W.Hill ex Maiden and *P. taeda* L. seed behavior under controlled deterioration and (2) estimating viability equations.

2. MATERIAL AND METHODS

This research work was carried out at the Laboratory of Post-harvest Technology, Faculty of Agricultural Engineering, Campinas State University, Brazil, from 2000 to 2003. *E. grandis* and *P. taeda* seed lots were harvested in 1999/2000 and 2000/2001 seasons, in Forest Experimental Stations of Anhembi and Itapetinga, São Paulo State, respectively.

E. grandis physical purity analysis was performed using a 5-g-working sample, which was separated in pure seeds, other seeds and inert matter. The 1,000-seed weight determination for both species was carried out using eight 100-pure seed sub samples (ISTA, 2006).

Lipid content was detected in 2-g-ground seed samples, placed in Whatman paper cartridges (80 mm length x 33 mm of internal diameter); the extraction was performed with ether of petroleum in a Soxhlet apparatus for 5 h and the amount of lipids was determined gravimetrically (AOAC, 1995). Activity of water was carried out at $25 \pm 0.3^\circ\text{C}$ on three replicates of ground seeds for each mc, using the chilled-mirror dew point technique.

Seed mc were adjusted to eight levels from initial values, using desiccators with silica gel or through rehydration over water (4 cm), both at 25°C . Seed sub samples were weighed periodically for controlling the amount of removed or absorbed water, until desired mc had been achieved. After a 4 day-period for stabilization at 4°C in sealed containers, seed mc (% f.wt.) was determined at $130-133^\circ\text{C}$ during 2 hours for each mc (ISTA, 2006), using three 5 g sub samples of intact (*E. grandis*) and ground seeds (*P. taeda*) and the values recorded are listed in Table 1.

Table 1 = Seed moisture content analyzed for the species.
Tabela 1 = Graus de umidade de sementes analisados para as espécies.

<i>E. grandis</i>	<i>P. taeda</i>
1.2	1.5
3.0	4.0
4.7	5.7
7.5	8.0
11.3 *	12.9 *
14.4	13.5
16.3	17.0
18.1	19.5

* Initial mc values Graus de umidade iniciais.

Seeds were sealed in laminated paper (polyester / aluminium / low density polyethylene, with total thickness of 65 µm) with a minimum of 10 x 0.6 g sub samples for *E. grandis* and 10 x 200 seed sub samples for *P. taeda* for each combination mc / T and stored at 40, 50 and 65 (± 0.5°C), until complete survival curves were achieved (Table 2).

E. grandis seed germination tests were performed using 4 x 0.15 g sub samples; for *P. taeda*, a previous dormancy releasing through stratification was proceeded (5°C, 28 days), using 8 x 25 seed sub samples. Seed sub samples were placed over a moistened filter paper in 11 x 11 cm plastic boxes and kept in an incubator under alternate temperature regime (20-30°C, 16-8 hours, 40 W white light at 30°C); seedling counts were recorded at 7 and 14 days (*E. grandis*) and at 7 and the final one at 28 days (*P. taeda*). Seeds were considered germinated according to the criterion of the radicle protrusion (SOUZA and CARDOSO, 2000).

Seeds with mc below 6% were previously rehydrated at 25°C during 48 hours over a nylon screen in a plastic box with 1.5 cm of distilled water on the bottom, aiming at avoiding predictable damages due to fast rehydration.

The origin of the survival curves on the Y-axis doesn't converge to the same point, requiring a statistical adjustment in order to get the same value (K_i) with the smallest possible deviation. The germination percentage values were plotted in X-Y graphs and regression lines were fitted to probit values through Y-axis scale transformation. The residual deviances for survival curves at different T were analyzed to give

Table 2 – *E. grandis* and *P. taeda* seed mc analyzed during storage at 40, 50 and 65°C.

Tabela 2 – Graus de umidade de sementes de *E. grandis* e *P. taeda* analisados durante o armazenamento a 40, 50 e 65 °C.

Storage temperature (°C)		Temperatura de armazenamento (°C)			
		<i>E. grandis</i>		<i>P. taeda</i>	
40	50	65	40	50	65
	1.2	1.2	1.5	1.5	1.5
	3.0	3.0	4.0	4.0	4.0
	4.7	4.7	5.7	5.7	5.7
7.5	7.5	7.5	8.0	8.0	8.0
11.3	11.3	11.3	12.9	12.9	12.9
14.4	14.4	14.4	13.5	13.5	13.5
16.3	16.3	16.3	17.0	17.0	17.0
18.1	18.1	18.1	19.5		

the best fit to the viability equation, using the multiple regression analysis within the generalized linear model (HAY et al., 2003).

The specific constants of each species were achieved using decimal logarithms of sigma (frequency of seed deaths distribution in time or the number of days required for seed germination drop by one probit) and mc for each combination mc / T. Seed germination with or without rehydration was analyzed through a randomized statistical design.

3. RESULTS AND DISCUSSION

Seed physical pure percentages and 1,000-seed weights detected were 19.0% and 0.226 g (*E. grandis*) and 100.0% and 25.0 g (*P. taeda*). Extraction relationship or fruit / seed relationship is the amount of capsules required to produce one kg of mixed seeds and presented a medium rate of 15:1 for *E. grandis*. Taking into account those values, the numbers of pure seeds / kg were estimated in 841,643 and 40,000 for *E. grandis* and *P. taeda*, respectively, which is similar to the result reported by Vieira and Diniz, 1995 (± 700,000 / kg, *E. grandis*). According to Gentil and Ferreira (2002), the 1,000-seed weight is affected by seed size uniformity, foundation plants and environmental factors.

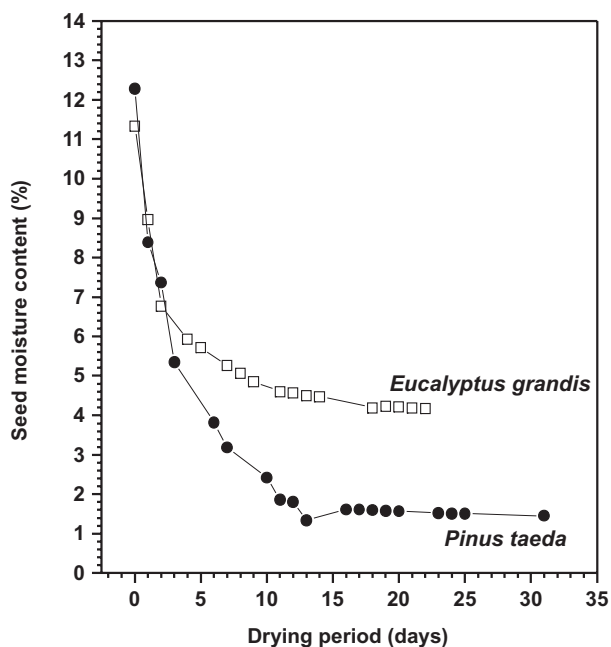


Figure 1 – Seed drying rates over silica gel at 25°C for *E. grandis* and *P. taeda*.

Figura 1 – Taxas de secagem de sementes sobre sílica gel a 25 °C de *E. grandis* e *P. taeda*.



Seed lipid contents recorded in *E. grandis* and *P. taeda* were 24.2 and 52.3%, which were higher than those achieved for *Dalbergia nigra* and *Dimorphandra mollis* (7.0% and 14%), respectively (CHAVES and USBERTI, 2004)

E. grandis seeds lost water faster over silica gel after 48-h-drying, *E. grandis* and *P. taeda* presented seed mc around 6.8 and 7.4% (Figure 1), probably due to the different lipid contents (BROOKER et al., 1992). Seed mc for both species presented an inverse relationship with drying period. The drying process over silica gel revealed positive results for the species and the lowest mc were obtained without damages to the seeds.

No statistical differences were recorded for *E. grandis* seed germination among mc (Table 3); however the highest and lowest percentages were obtained with 7.5 / 11.3% mc (no rehydration) and with 1.2% mc (with rehydration), emphasizing the need for rehydration before germination test for mc lower than 6%. Lowest *P. taeda* seed germination percentages were recorded with 8.0 and 1.5% mc, unrelated to rehydration however seeds with 12.9% mc presented a significant increase in germination after rehydration. Bradford (1995) reported that seed germination normally increases after rehydration.

Table 3 – Seed germination (%) of *E. grandis* and *P. taeda* after drying over silica gel.

Tabela 3 – Germinação de sementes (%) de *E. grandis* e *P. taeda* após a secagem sobre sílica-gel.

Seed mc	<i>E. grandis</i>	
	No rehydration Sem reidratação	With rehydration Com reidratação
11.3	87.5 a	82.5 a
7.5	84.5 a	70.5 a
4.7	78.0 a	79.0 a
3.0	68.0 a	80.0 a
1.2	68.0 a	77.0 a
<i>P. taeda</i>		
12.9	82.0 a B	95.0 a A
8.0	75.5 ab A	78.5 b A
5.7	81.0 a A	85.0 ab A
4.0	85.0 a A	89.0 ab A
1.5	63.0 b A	74.0 b A

Means followed by different lower case letters in the columns and upper case letter in the lines, are statistically different at $p < 0,05$.

Médias seguidas por diferentes letras minúsculas nas colunas e maiúsculas nas linhas são estatisticamente diferentes a $p < 0,05$.

Seeds of *P. taeda* presented a lower equilibrium relative humidity (erh) than *E. grandis*, when stored in similar conditions. Oily seeds present erh lower than starchy ones, when exposed to similar environments, since they don't absorb or absorb less water, due to being hydrophobic.

Seed longevity increased logarithmically according to reduction of mc until all weak bound water had been removed. The inflection points of the desorption isotherms for *E. grandis* and *P. taeda* (4.9% mc, erh at 22.9% and 4.1% mc, erh at 21.2%, Figure 2) mean the discontinuity of the inverse relationship between longevity and mc or the lowest mc limits for application of the viability

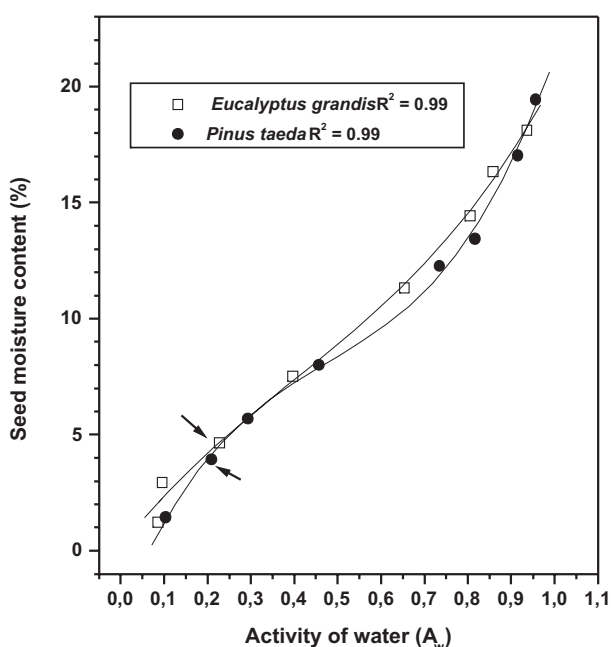


Figure 2 – Relationship between seed mc (% f.wt.) and activity of water (%) of *E. grandis* and *P. taeda*, at 25 °C. The curves represent sorption and desorption isotherms, above and below the initial mc values (11.3 and 12.9%, respectively). The arrows point out the inflection points of the isotherms (4.9 and 4.1% mc), which mean the lowest mc limit for application of the viability equations at 65 °C.

Figura 2 – Relações entre grau de umidade (% b.u.) e atividade de água (%) de *E. grandis* e *P. taeda* a 25 °C. As curvas representam isothermas de sorção e desorção, acima e abaixo dos valores iniciais de graus de umidade (11,3 e 12,9%, respectivamente). As setas apontam para os pontos de inflexão das isothermas (graus de umidade de 4,9 e 4,1%), que indicam os limites inferiores de grau de umidade para a aplicação da equação de viabilidade a 65 °C.

equation at 65°C. Those values have already been estimated for another species with variable results, as follows: 2.4% mc for peanuts (USBERTI and GOMES, 1998); 6.2% mc for peas (ELLIS et al., 1989); 2.9 and 4.0% mc for *D. nigra* and *D. mollis* (CHAVES and USBERTI, 2004) and 3.6% mc for cottonseed (USBERTI et al., 2006).

Probit seed survival curves for the species after storage at 40, 50 and 65°C are displayed in Figure 3, showing the effects of mc and T on seed storability. When mc and T were reduced, predictable seed longevity increases were observed. Ellis (1984) reported that seed survival curves always present the same format, differing among them just in relation to the deterioration period,

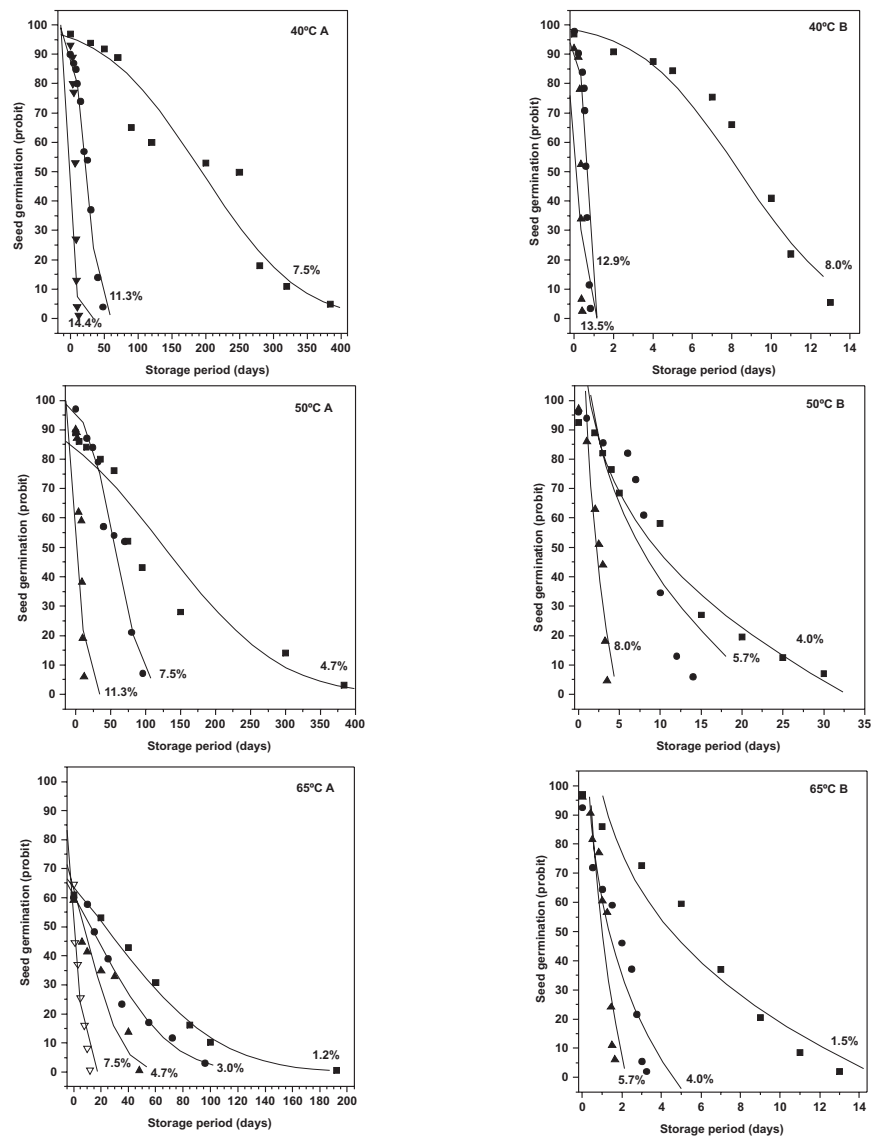


Figure 3 – Seed survival curves (probit) for *E. grandis* (A, on the left) and *P. taeda* (B, on the right), stored at 40, 50 and 65°C. Symbols and straight lines mean germination percentages and survival curves, respectively.

Figura 3 – Curvas de sobrevivência de sementes (em probit) para de *E. grandis* (A, à esquerda) e *P. taeda* (B, à direita), após o armazenamento a 40, 50 e 65 °C. Símbolos e retas significam porcentagens médias de germinação e curvas de sobrevivência, respectivamente.

but presenting the same standard deviation. In the present study the sigmoid shape of the survival curves was strongly affected by temperature and mc increase especially for *P. taeda* seeds.

Relationship differences between mc and longevity were observed and *P. taeda* seed sensitivity to mc was higher than *E. grandis*. Tompsett (1986) worked with two arboreal species and verified that *Ulmus carpinifolia* Borkh. seed was significantly more sensitive to mc than *Terminalia brassii* Exell.

Seeds of *E. grandis* stored at 40°C with 7.5% mc had a sigma of 90 days, while for *P. taeda* with 8.0% mc that value was reduced to 5 days (Figure 3). The combination of highest T and mc resulted in seed longevity reductions, confirming the tremendous effect of mc on seed longevity.

E. grandis seeds stored at 50°C with 7.5% mc presented a sigma of 40 days, while for *P. taeda* with 8.0% mc that value was reduced to 2 days (Figure 3). In seed samples with 4.7 and 4.0% mc, the calculated sigma values were 55 and 4 days, respectively. Almeida et al., 2005, working with three *Pinus* species, reported that *P. taeda* seed germination was not affected after a 2-minute-storage at d" 50°C.

Seeds of *E. grandis* stored at 65C with 1.2 and 7.5% mc presented sigma values of 40 and 1 days, respectively; however in *P. taeda* with 1.5% mc that time was 3 days (Figure 3). For seed germination to reach values lower than 5%, with 3.0 and 4.0% mc, 96 and 3.25 days were required, respectively. *P. taeda* seed germination reduction was higher than for *E. grandis* throughout the experiment, since oily seeds deteriorate faster than starchy ones.

Seed mc and sigma values after storage at 40, 50 and 65°C are presented in Table 4. *E. grandis* seeds with 18.1% mc stored at 40 and 50°C presented sigma values of 0.4 and 0.1 day; in *P. taeda* with 17.0% mc stored at 40 and 50°C, those values decreased to 0.009 and 0.002 day.

Constraining 24 survival curves to the same origin point on the Y-axis yielded different *Ki* values for *E. grandis* and *P. taeda* (1.351 and 1.819; 90 and 98% seed germination). Significant effects for log mc and T were observed while non-significant ones were calculated for the interaction log mc / T ($F = 0.47$ *P. taeda*; 3.08 *E. grandis*).

Better statistic adjustments to estimate viability constants were achieved after removal of the lowest mc results at 65 and 50°C (1.2, 3.0 and 4.7% mc for

Table 4 – Seed mc (%) and sigma (days) for *E. grandis* and *P. taeda*. stored at 40, 50 and 65°C.

Tabela 4 – Graus de umidade (%) e sigma (dias) de sementes de *E. grandis* e *P. taeda*, armazenadas a 40, 50 e 65 °C.

Mc	Sigma (days)					
	<i>E. grandis</i>			<i>P. taeda</i>		
	40 °C	50 °C	65 °C	40 °C	50 °C	65 °C
1.2	-	-	43.19	-	-	-
1.5	-	-	-	-	-	3.29
3.0	-	-	27.81	-	-	-
4.0	-	-	-	-	7.35	0.97
4.7	-	97.27	14.51	-	-	-
5.7	-	-	-	-	4.59	0.6
7.5	137.6	40.83	3.59	-	-	-
8.0	-	-	-	4.7	1.27	0.15
11.3	17.33	4.97	0.44	-	-	-
12.9	-	-	-	0.3	0.06	0.006
13.5	-	-	-	0.15	0.023	0.005
14.4	4.2	0.84	0.13	-	-	-
16.3	0.76	0.29	0.03	-	-	-
17.0	-	-	-	0.009	0.002	-
18.1	0.4	0.1	-	-	-	-
19.5	-	-	-	0.001	-	-

E. grandis; 1.5 and 4.0% mc for *P. taeda*). The constants K ($K = K_E - C_H t - C_Q t^2$) obtained define the equations at 40, 50 and 65°C, as follows: *E. grandis*: $K = 8.084$; 5.745; 2.486 and $C_W = 6.641$; 5.103; 2.777; *P. taeda*: $K = 8.802$; 4.673; 1.454 and $C_W = 8.748$; 5.596; 2.949.

The constants K_E and C_W estimated for *E. grandis* (9.661 and 6.467) were different from those calculated for *P. taeda* (8.838 and 5.981), confirming the observation done by Ellis (1984) that for cereal seeds those values are higher than oily seeds, indicating the need for drying those seeds to lower mc levels. The constants that reflect seed sensitivity to T (C_H and C_Q) were 0.03498 and 0.0002330 (*E. grandis*) and 0.10340 and 0.0005476 (*P. taeda*).

The viability equation constants for *E. grandis* appear to be close to that reported for cottonseed ($K_E = 9.240$, $C_W = 5.190$, $C_H = 0.03965$ and $C_Q = 0.000426$) (USBERTI et al., 2006). An inverse relationship was observed between mc and seed longevity for both species.

The viability equations estimated for *E. grandis* and *P. taeda* seeds were:

$$v = Ki - p/10^{9.661-6.467.\log m-0.03498t-0.0002330t^2} \quad E. grandis$$

$$v = Ki - p/10^{8.838-5.981.\log m-0.10340t-0.0005476t^2} \quad P. taeda$$

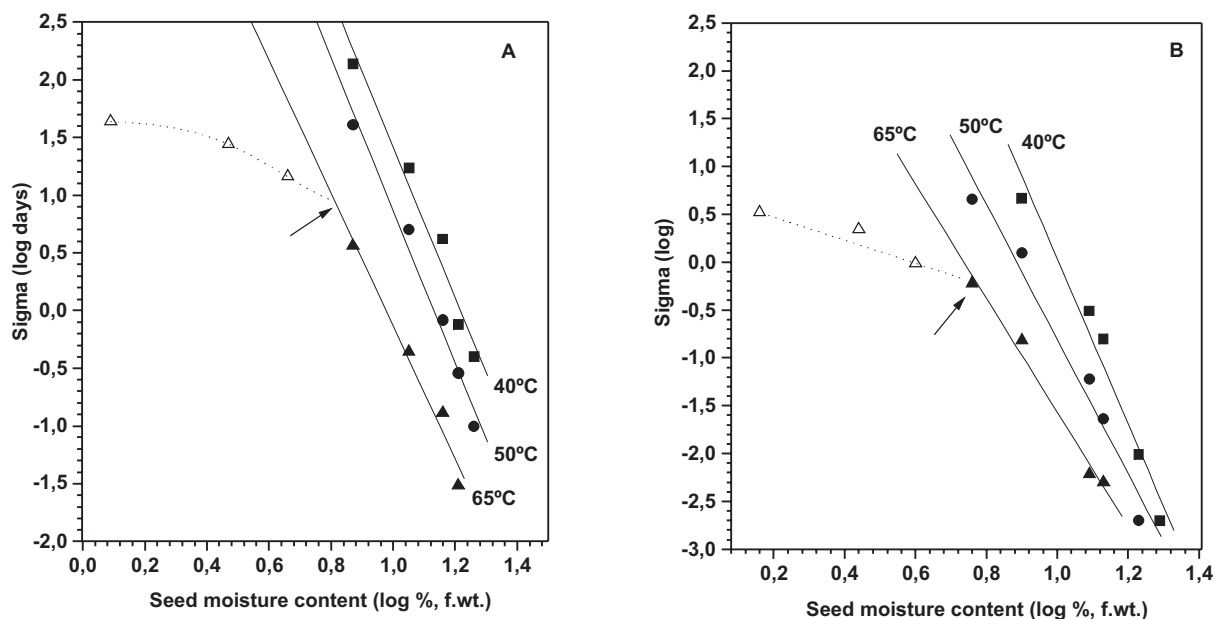


Figure 4 – Relationship between the logarithm of seed mc and the logarithm of sigma for *E. grandis* (A, on the left) and *P. taeda* (B, on the right), stored at 40, 50 and 65°C. Solid lines represent slopes, which are constrained to one common value. The dotted lines show different trends at 65°C, below the lower mc limit for application of the viability equation, pointed out by the arrows.

Figura 4 – Relações entre o logaritmo de grau de umidade da semente e o logaritmo de sigma para de *E. grandis* (A, à esquerda) e *P. taeda* (B, à direita), após o armazenamento a 40, 50 e 65 °C. Linhas sólidas representam curvas, após serem direcionadas a um ponto comum. As linhas pontilhadas mostram diferentes tendências a 65 °C, abaixo do limite inferior de grau de umidade para a aplicação da equação de viabilidade, indicados indicado pelas setas.

Figure 4 shows the logarithmic relationship between seed mc and sigma at 40, 50 and 65°C and reveals the parallelism among the straight lines, due to different T used. Dickie et al. (1990) reported that the viability equation could be applied from -13 to 90°C and also observed a negative relationship between C_w values and seed oil content, among different species.

Medeiros et al. (1998), using $K_E=7.5498$ and $C_w=3.76$ and adopting $C_H=0.0329$ and $C_O=0.000478$ as universal constants, estimated the sigma value of 1,167 years for the species *Astronium urundeuva* (Fr.All.) Engl. stored at -20°C with mc in equilibrium at 15°C and 15% RH.

Using the viability equations and adopting 4°C (domestic refrigerator) and 7.5 and 8.0% mc (erh at 40 and 45%), achieved in a 2-day drying period over silica gel, it is expected that *E. grandis* and *P. taeda* seeds would present sigma values of 19.9 and 2.9 years. That different performance could be due to the different chemical composition of those seeds, since *P. taeda* presented higher lipid content.

4. CONCLUSIONS

. *P. taeda* and *E. grandis* presented, respectively, sub- and true orthodox seed behaviour to storage.

. *P. taeda* revealed erh and seed storability lower than *E. grandis*.

. Lowest limits for application of the viability equation at 65°C were 4.9 and 4.1% mc for *E. grandis* and *P. taeda*, respectively.

5. ACKNOWLEDGEMENTS

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