Deterioration rates of brazilwood seeds (*Caesalpinia echinata* Lam.) under high temperatures

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ABSTRACT - (Deterioration rates of brazilwood seeds (*Caesalpinia echinata* Lam.) under high temperatures). *Caesalpinia echinata* seeds deteriorate in less than three months at ambient temperature, although they can remain viable for up to five years when kept under freezing temperatures. We studied the deterioration of brazilwood seeds when submitted to different conditions of temperature and humidity aiming to check the applicability of mathematical models for predicting loss of viability of those seeds. The results showed that the high temperatures preconized for the rapid deterioration and simulation of storability produced an irregular behavior, suggesting that the deteriorative metabolism of these seeds may not correspond to the metabolism during the natural storage. Applying the equations, seeds with 8% water content stored at 7 °C could be stored for five years, while the literature shows that these seeds would not tolerate more than a year. On the other hand, the prediction of storage with 10% of water content at -18 °C would be, by these equations, 128 years, while the literature shows that seeds under these conditions would lose viability in less than ten years. The results showed that the current models might present restrictions on their application, depending on the species and conditions.

Keywords: predictability equation, seed conservation, viability

Introduction

The seed longevity, which determines the storage potential, is hugely changeable according to species and genetically determined. Nevertheless, environmental factors and storage conditions influence this longevity (Barbedo *et al.* 2013). The suitable storage allows the conservation of genetic resources of the natural population through seeds. However, the viability preservation depends on seed behavior knowledge, dealing with the storage to which they are submitted (Chaves & Usberti 2003). After shedding, seeds that do not start germination will inevitably initiate a process of deterioration. This is determined by a series of physiological, biochemical, physical and cytological changes, in progressive rhythm, leading to the decrease of vigor and germination capacity, culminating in the death of the seed (Marcos-Filho 2015). As the seeds deteriorate, the membranes become less selective, respiratory and biosynthetic
activities are reduced, and the germination becomes slower, more uneven and more sensitive to temperature changes. Furthermore, the emergence of seedlings becomes more sensitive to adversities and at the end of the process the seeds lose even the ability to initiate germination (Delouche & Baskin 1973). The speed of these changes depends, among other factors, on the ambient temperature and on the water content of the seeds (Roberts 1973). Seeds can deteriorate faster, for example, when the ambient temperature is higher and/or when they maintain a high water content (Marcos-Filho 2015).

The prediction of viability loss of stored seeds is important both for the maintenance of germplasm and for the management of seed storage (Sinício et al. 2009). Since the half past century studies have been developed in order to predict the viability loss, with survival curves and relationships between temperature, humidity and some measure of viability (Pritchard & Dickie 2003). Models developed by Roberts (1973) and Ellis & Roberts (1980) are still used to estimate the longevity of the stored seeds, mainly for the crop species. Those authors proposed study models in order to obtain the constants of the equations. These constants can be obtained, in the Roberts (1973) model, through four germination tests applied to seeds stored in three different conditions, including two degrees of seed water content and two ambient temperatures. Yet in the improved model by Ellis & Roberts (1980), which is more complex and includes the initial quality of the lot, several temperatures and water contents are evaluated. In the Millenium Seed Bank, of Royal Botanic Garden (Kew, UK), there is a protocol to determine the longevity of the seeds of each species. This is based on the artificial aging of the seeds in a condition of 60% of relative humidity and 45 °C (Newton et al. 2009). This temperature is used in Caesalpinia echinata Lam. (Leguminosae - Caesalpinoideae), for example, for the drying of the seeds after the harvest aiming at the rapid reduction of the water content to values below 10% (Barbedo et al. 2002); a lower or higher temperature can be used for the seed lots with, respectively, lesser or greater estimated longevity. The advantage of using high temperatures is that once it results in faster deterioration of the seeds, these evaluations could be performed in much less time.

Although those models have been developed on the basis of the typically orthodox seeds behavior of crop species, some studies were carried out for brazilian native species, such as Astronium urundeuva (Fr. All.) Engl. (Medeiros 1996), Dalbergia nigra (Vell.) Fr. All. ex Benth.) (Chaves 2001), Dimorphandra mollis Benth. (Chaves & Usberti 2003), Balfourodendron riedelianum (Engler) Engler (Ignácio 2013), Cedrela odorata L., Ceiba petandra (L.) Gaertn., Dalbergia spruceana Benth. and Handroanthus albus (Cham.) Mattos (Lima Jr. & Ellis 2005, Lima Jr. et al. 2005).

Nonetheless the models are applied and have their immediate utility for the management of gene banks as well as the use of stored seeds, there is few information about the factors that change the constants of the equations. As previously described, several are the factors that contribute for higher or lower speed of deterioration of the stored seeds, but few are effectively studied scientifically. Roberts (1973) recommends that experiments should be carried out with different genotypes of the same species to define genotypic effects in terms of its influence over the constants of the basic viability equations.

Caesalpinia echinata seeds are an interesting model for storage and deterioration studies, mainly because of their atypical behavior for the protocol standards. The first studies with the storage of these seeds demonstrated that they deteriorate in less than three months when stored at temperatures close to that found in their habitat; however, they can remain viable for up to two or five years in, respectively, cooling (Barbedo et al. 2002) and freezing (Mello et al. 2013) temperatures.

In this study, we analyzed the relationship between the storage conditions of C. echinata seeds, specially the ambient temperature and the water content of the seeds, and their capacity to maintain viability, aiming to help the development of the viability equations and management of germplasm banks. We also evaluated the applicability and reliability of current simulation models of loss of viability under different storage conditions. For this, after obtaining the predictability equations, different storage conditions were used, comparing the simulated data with the real ones described in the literature for C. echinata seeds (Barbedo et al. 2002, Mello et al. 2013).

Material and methods

Obtaining and characterizing plant material – The Caesalpinia echinata seeds were collected in Paulinia (22°46'6"S, 47°09'8"W, 620 m of altitude, hereinafter named PA), in 2014, São Paulo (23°38'8"S, 46°38'8"W, 860 m of altitude, named SP), in 2014, and Mogi
Guaçu (22°13'S, 46°33'W, 640 m of altitude), in 2013 and 2014 (respectively named M1 and M2), in order to obtain seeds produced under different soil and climatic conditions (table 1). The three localities have Cwa climate according to Köppen classification. Seeds from mature fruits collected from 15 to 25 trees, as well as those freshly dispersed (on the same day of collection, in order to get a sufficient number of seeds for treatments), composed the lot of each source. Mature fruits were manually picked, according to the visual description of Borges et al. (2005), and exposed to the sun in covered trays with shading screen, for their spontaneous opening. After the manual extraction, the seeds remained in the trays on the lab bench, for drying at room temperature, for about ten days. Then were packed in paper bags and stored in the freezer, at -18 °C. The seeds were initially characterized by its water and dry matter content, and germination, as described thereafter. Shading screens were laid on the ground to obtain the recently dispersed seeds (less than 24 h), following the same procedures of drying, packing and storage described for seeds of mature fruits.

Table 1. Distribution of the aging treatments of *Caesalpinia echinata* Lam, seeds according to their origin (M1: Mogi-Guaçu, harvest 2013; M2: Mogi-Guaçu, PA: Paulínia; SP: São Paulo, harvest 2014), incubation periods (p1 to p6) at temperatures (T) of 40 °C and 50 °C, and target water contents (WC) of 7%, 11% or 15%.

| Origin | WC (%) | T (°C) | Incubation periods (h) |
|--------|--------|--------|------------------------|
|        |        |        | p1 | p2 | p3 | p4 | p5 | p6 |
| M1     | 7      | 40     | 12 | 72 | 120 | 240 | 480 | 600 |
|        |        | 50     | 12 | 72 | 96  | 120 | 240 |  |
|        | 11     | 40     | 12 | 72 | 240 | 480 | 720 |  |
|        |        | 50     | 12 | 24 | 36  | 48  | 72  | 120 |
|        | 15     | 40     | 6  | 12 | 24  | 36  | 48  |  |
|        |        | 50     | 6  | 12 | 24  | 36  | 48  | 60 |
| M2     | 7      | 40     | 12 | 72 | 120 | 240 | 480 | 600 |
|        |        | 50     | 12 | 72 | 96  | 120 | 240 |  |
|        | 11     | 40     | 24 | 72 | 240 | 480 | 720 |  |
|        |        | 50     | 24 | 36 | 48  | 60  | 72  |  |
|        | 15     | 40     | 6  | 12 | 24  | 48  | 72  |  |
|        |        | 50     | 6  | 12 | 24  | 48  | 60  |  |
| PA     | 7      | 40     | 24 | 72 | 240 | *   | *   | *   |
|        |        | 50     | 24 | 72 | *   | *   | *   | *   |
|        | 11     | 40     | 24 | 72 | 240 | 480 | *   | *   |
|        |        | 50     | 24 | 48 | 72  | 96  | 120 |  |
|        | 15     | 40     | 24 | 72 | *   | *   | *   | *   |
|        |        | 50     | 24 | 48 | *   | *   | *   |  |
| SP     | 7      | 40     | 24 | 72 | *   | *   | *   | *   |
|        |        | 50     | 24 | 72 | *   | *   | *   |  |
|        | 11     | 40     | 24 | 72 | 240 | 480 | *   | *   |
|        |        | 50     | 24 | 72 | 120 | 240 | *   | *   |
|        | 15     | 40     | 24 | 72 | *   | *   | *   |  |
|        |        | 50     | 24 | 72 | *   | *   | *   |  |

* These treatments were not carried out because seeds did not germinate in the last evaluation or because there were no remaining seeds.
Evaluation of the physical and physiological quality of seeds - Seed water content was obtained by drying at 103 ± 2 °C during 17 h, according to the methodology of brazilian rules for seed analysis (Brasil 2009), with the results registered on wet basis. Three replicates of five seed per batch were used.

The germination tests were performed on germination paper rolls, at 25 °C and continuous light, according to the germination characteristics of this species, described by Mello & Barbedo (2007), with four replicates of ten seeds. The paper rolls were packed in perforated polyethylene bags and placed inside Mangelsdorff-type germinators, with 100% relative humidity provided by maintaining water at the base of the germinator. Before seeding, the seeds were immersed in a 0.2% benomyl solution (systemic fungicide) for 10 seconds, then superficially dried with absorbent paper and powdered with non-systemic captan (0.5 mg seed⁻¹) fungicide (Lisbôa-Padulla et al. 2009). Germination (0.5 cm of primary root protrusion) and the normal seedling development capacity (as described in Borges et al. 2005, hereinafter named normal seedling) were registered every three days until 30 days.

Seed deterioration - The seeds remained stored at -18 °C (Mello et al. 2013), in paper bags, during one year for the seeds collected in Paulínia, São Paulo and Mogi Guçu in 2014, and during two years for the seeds collected also in Mogi Guçu, in 2013. The seeds of each batch were divided into three sub-lots, in order to obtain treatments with three levels of seed water content (WC).

One third of the seeds of each batch remained with their initial values of WC (10-11%). Another third part of the seeds were subjected to drying in nylon bag placed inside the desiccator with silica gel, until the reduction of WC to about 7%. The last third part of each batch was subjected to hydration (through humidification in nylon bags on 100% relative humidity, in closed glass container) up to about 15% WC. At the end, water content of the seeds was determined, as described previously.

The seeds of each WC level from each lot, were divided into glass jars with hermetic closure (70 seeds per jar), and incubated at 40 °C and 50 °C, to obtain the complete curves of deterioration, based on the recommendations of Ellis & Roberts (1980) and used by Chaves & Usberti (2003). The treatments were constituted by increasing periods (until 30 days) of incubation inside the seed jars with each of the three hydration target levels (7%, 10% and 15%), in incubation chambers at temperatures of 40 °C and 50 °C, until the complete loss of viability, for each lot (table 1). At the end of each incubation period, a jar was withdrawn and the seed deterioration evaluated through germination test, as described previously. The equations of the straight lines resulting from linear regression allow to obtain the Kv values (constant of the species), C₁ (coefficient of humidity) and C₂ (coefficient of temperature), in order to estimate the time for half the lot to lose viability (p), by the equation: 
\[ \log p = K_v - C_1m - C_2t \] (Roberts 1973).

Experimental design and statistical analysis of the data - The experimental design for all the experiments was completely randomized. For the evaluation of the initial seed physiological quality, the obtained data were submitted to analysis of variance (F test, p < 0.05) and the means were compared by Tukey’s test at 5% (Santana & Ranal 2004). For the germination data under different deterioration conditions, means and standard deviations are shown. They were transformed into probit, to check the linear relationship between viability and the incubation periods, according to the methodology proposed by Roberts (1973) and Ellis & Roberts (1980).

**Results and Discussion**

Water content (WC), dry matter (DM), germination (G) and normal seedling (N), for initial characterization of the seeds of different origins, are shown in table 2. It can be observed that G and N were similar among the lots. WC after drying or hydration were mostly close to the target ones and the DM were also close among seeds of different origins.

The results from seed survival evaluation of each origin after incubation in different conditions (temperatures and WC, figures 1 to 8) showed that, the higher, both the WC and the temperature, the more pronounced the decrease in G and N, for all the lots. This is due to the great influence of the water inside the seeds in the processes of deterioration (Marcos-Filho 2015). However, increasing WC and incubation temperature did not change the speed of deterioration in the same way. For example, M1 seeds incubated with 7% to 50 °C (figure 1) maintained above 50% germination after 20 days, while M2 (same population, different years) had no germination under the same conditions (figure 2), probably indicating a higher degree of maturity of the former. This can also be verified in the incubation of the seeds with higher WC (11%, 40 °C, figures 1 and 2) with faster deterioration of M1 in relation to M2.
Table 2. Initial characterization of *Caesalpinia echinata* Lam. seed lots in terms of water content (WC, in %), initial and after the period to reach the target WC of 7% (T7) and 15% (T15), dry matter (DM, in g seed⁻¹), germination (G) and normal seedling development (N). M1: Mogi-Guaçu, harvest 2013; M2: Mogi-Guaçu, PA: Paulínia and SP: São Paulo, harvest 2014). Means with the same letter (lower case in columns, capital in the line) did not differ by Tukey’s test (5%).

| Lot   | WC (%) | DM (g seed⁻¹) | G (%) | N (%) |
|-------|--------|---------------|-------|-------|
|       | Initial | T7  | T15 |       |       |
| M1    | 10.9 abB | 8.4 aC | 15.5 aA | 0.280 a | 83 a | 70 a |
| M2    | 11.3 aB | 8.6 aC | 15.1 aA | 0.248ab | 85 a | 70 a |
| PA    | 9.3 bB | 7.2 aC | 14.9 aA | 0.235 b | 87 a | 57 a |
| SP    | 10.8 abB | 5.7 bC | 13.3 bA | 0.267ab | 93 a | 80 a |

Another interesting aspect was the faster deterioration of the seeds of all origins in both incubation temperatures when the water content of the seeds was 15%, with almost null germination after three days. Even the seeds from SP, that still had 30% of germination (figure 4), did not produce normal seedlings (figure 7). It is also showed that the progression of the germination decrease of those seeds tends to nullity before the fourth day (figure 4).

Despite the differences pointed out previously, in general it is noted a certain pattern of the incubated seeds behavior with different water content and at different temperatures. This fact would allow enough uniformity for the establishment of deterioration patterns of the species seeds, as expected by Roberts (1973) and Ellis & Roberts (1980). This would allow one to obtain the coefficients of the viability equations, consequently, the estimates of longevity in storage into certain temperature, with certain water content.

According to Roberts (1973), from the controlled deterioration of the seeds in three different conditions of water content and temperature, it would be possible to obtain the species coefficients and, consequently, its viability equation, since at least two temperatures and two water contents have been provided. Initially, data must be transformed into probits, making the sigmoid of deterioration a straight line; then, estimating both the average germination drop to 50% and its respective standard deviation on the three obtained equations with the three deterioration conditions, it is possible to obtain the species (Kᵥ), water content (Cₑ) and temperature (Cₜ) coefficients and, therefore, the viability equation: \( \log \ p = Kᵥ - Cₑ m - Cₜ t \) (more details in Newton et al. 2009). In the present study, in which six different deterioration conditions were obtained, to four lots, different combinations of three conditions could be obtained, following recommendations of Roberts (1973). Thus, some combinations were analyzed, using the M1 lot. In the first simulation (S1), we used the combination of 7% WC at 40 °C, 7% WC at 50 °C and 11% WC at 40 °C. In the second simulation (S2), we used the combination of 7% WC at 40 °C and 50 °C, and 15% WC at 40 °C.

Then, the equations obtained for S1 and S2 were used for predicted viability decrease in two storage conditions: 8% WC at 7 °C (storage A) and 10% WC at -18 °C (storage B). These two conditions were chosen because they were close to the ones described in the literature for the species seed storage (Hellmann et al. 2008, Mello et al. 2013).

Using the S1 equation, in the storage A, the reduction in 50% of viability would occur after five years, while the results of Hellmann et al. (2008) and Mello et al. (2013) show that these seeds would not tolerate more than one year. In the storage B the equation predicted 50% viability after 128 years, while the results of Mello et al. (2013) show that the storage should be for no more than ten years. When the S2 equation was used, the viability would decrease to 50% after 12 years in the storage A and, in the storage B, after 518 years, that is a situation even more distant from the real one.

From our results, we verified the need for more detailed studies about the process related to the loss of germination capacity and of producing normal seedlings of the *Caesalpinia echinata* seeds. The *C. echinata* seed conservation in storage has been a challenge for the researchers concerned about the seed conservation. Currently, its geographic distribution is restricted to a few forest fragments, which makes it an endangered species (Rocha et al. 2014). Among the most important acts to reverse this situation, there is the conservation *ex situ*, in seed banks (Rocha et al. 2006). Nevertheless, the *C. echinata* seed storability is still far from those of classical orthodox seeds, despite the fact that significant progress has been made. Initially, the lifespan of those seeds was considered short, tolerating no more than three months in natural storage.
Figure 1. Germination (G%) of *Caesalpinia echinata* Lam. seeds, harvested in Mogi-Guaçu (SP) in 2013 and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 h. Values are means ± SD.
Figure 2. Germination (G%) of *Caesalpinia echinata* Lam. seeds, harvested in Mogi-Guaçu (SP) in 2014 and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 h. Values are means ± SD.
Figure 3. Germination (G%) of *Caesalpinia echinata* Lam. seeds, harvested in Paulinia (SP) in 2014 and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 h. Values are means ± SD.
Figure 4. Germination (G%) of *Caesalpinia echinata* Lam. seeds, harvested in São Paulo (SP) in 2014 and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 h. Values are means ± SD.
Figure 5. Development of normal seedlings (N%) of *Caesalpinia echinata* Lam., seeds harvested in Mogi-Guaçu (SP) in 2013, and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 hours. Values are means ± SD.
Figure 6. Development of normal seedlings (N%) of *Caesalpinia echinata* Lam., seeds harvested in Mogi-Guaçu (SP) in 2014, and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 hours. Values are means ± SD.
Figure 7. Development of normal seedlings (N%) of *Caesalpinia echinata* Lam., seeds harvested in Paulínia (SP) in 2014, and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 hours. Values are means ± SD.
Figure 8. Development of normal seedlings (N%) of *Caesalpinia echinata* Lam., seeds harvested in São Paulo (SP) in 2014, and incubated at 40 °C or 50 °C with target water content of 7%, 11% or 15%, until 720 hours. Values are means ± SD.
(Barbedo et al. 2002). Later, important advances were obtained with the application of suitable techniques, such as the harvest at the physiological maturity point (Borges et al. 2005), the control of drying (Martini-Neto & Barbedo 2015) and the storage, including the freezing (Hellmann et al. 2006), fungus control (Lisbôa-Padulla et al. 2009, Padulla et al. 2010) and monitoring the chemical composition changes (Garcia et al. 2006, Borges et al. 2006, Hellmann et al. 2008, Mello et al. 2011), extending storage for up to five years (Mello et al. 2013). However, this storage period is not enough to allow its inclusion in germplasm banks. Therefore, it is necessary much more studies to understand the rapid deterioration of these seeds.

As shown by Lamarca & Barbedo (2012) and Martini Neto et al. (2014), the higher the temperature the more intense the deterioration of brazilwood seeds. There is also a strong correlation between brazilwood seed deterioration and the increase in the $O_2$ consumption without equivalent release of $CO_2$. This suggests that the deterioration of those seeds derives from oxidative processes, inhibited only when stored in freezing temperature. Nevertheless, the high temperatures analyzed by those authors were still within the limits found by the species in its natural environment. In the presente work, in which those limits were overcome, the rate of deterioration has been intensified and in an irregular way. This fact suggests that the deleterious seed metabolism under the conditions to which they were submitted for the storage simulation, may not correspond to the metabolism during the natural storage. Roberts (1973), who proposed the viability equations, had already alerted to the needs of studying and including the oxygen factor in the studies.

The estimation of the viability in storage is a fundamental tool for the management of germplasm banks. It has been applied with great sucess to the seeds of crop species. However, it has also been applied for different non-domesticated species, including Leguminosae trees natives from Brazil, as *Dimorphandra mollis* (Chaves & Usberti 2003), *Dalbergia nigra* (Chaves 2001) and *Dalbergia spruceana* (Lima Jr & Ellis 2005). In this work, the great variation of the results would be unacceptable for the estimation of the brazilwood seed longevity and, consequently, for the planning and management of those seeds in germplasm bank, indicating that the use of the predictability model must be done carefully, because not always fit for all species. The high temperatures used may have completely changed the metabolism, specially respiratory and oxidative and, consequently, the deterioration rates, interfering in the reliability of the equations of predictability. Therefore, the current models, that do not include respiration and oxidation process and, particularly the model proposed by Ellis & Roberts (1980), that uses the temperatures outside the natural occurrence range of the species, may present restrictions on their application, depending on the species and the conditions, as demonstrated for the predictability of brasilwood seed conservation. New studies should be carried out aiming the suitability of these models to the behavior and metabolism of *Caesalpinia echinata* Lam. seeds, species that gave the name to our country.

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