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## SLOW AND FAST DRYING IN SEEDS OF *Ocotea puberula* (Rich.) Ness<sup>1</sup>

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**ABSTRACT** – The objective of this work was to evaluate the influence of types of drying on seeds' quality, as recalcitrant, *Ocotea puberula* and determine their degree of critical humidity and lethal degree. Seeds harvested in Brunópolis, SC, with an initial moisture content of 38%, were dried to 18%, with gradients of 2%; using the equation of target weight to ensure that gradient in the stove (35 oC) and in a desiccator with silica gel (25 oC). After drying, the seeds were evaluated for water content, percentage and germination speed index (GSI), tetrazolium and T50. It was observed that up to 32% water content did not change in seed quality, regardless of the type of drying and verified significant loss of germination after this value. We conclude that the type of drying does not affect the quality of the seed; however, because it is a recalcitrant seed, reducing the water content below 32% decreased germination, and its degree of critical humidity and seeds with 22% moisture content had no germination in this study is the degree of lethal humidity of this species.

Key-words: Canela-guaicá; Recalcitrant seed; Water content.

## SECAGEM LENTA E RÁPIDA EM SEMENTES DE *Ocotea puberula* (Rich.) Ness

**RESUMO** – Objetivou-se avaliar a influência de tipos de secagem na qualidade de sementes, consideradas recalcitrantes, de *Ocotea puberula* e determinar o seu grau de umidade crítico e grau letal. Sementes colhidas em Brunópolis, SC, com teor de água inicial de 38%, foram secas até 18%, com gradientes de 2%; utilizando a equação de peso alvo para garantir este gradiente, em estufa (35 oC) e em dessecador com sílica-gel (25 oC). Após cada secagem, as sementes foram avaliadas quanto ao teor de água, porcentagem e velocidade (IVG) de germinação, T50 e tetrazólio. Foi observado que até 32% de teor de água não houve alteração na qualidade das sementes, independente do tipo de secagem, sendo verificada significativa perda de germinação após esse valor. Conclui-se que o tipo de secagem não influencia na qualidade dessas sementes; porém, por tratar-se de uma semente recalcitrante, a redução do teor de água abaixo de 32% prejudica a germinação, sendo seu grau de umidade crítico e sementes com 22% de teor de água não tiveram germinação, sendo neste estudo o grau de umidade letal desta espécie.

Palavras-chave: Canela-guaicá; Semente recalcitrante; Teor de água.

## 1. INTRODUCTION

Seeds are commonly classified according to their longevity, and tolerance to both desiccation and low temperatures, which can be either orthodox (tolerant) or recalcitrant (intolerant) (ROBERTS, 1973). However, Ellis et al. (1990) defined a third category as the intermediates, which are relatively desiccant-tolerant. Nonetheless, these seeds do not withstand low temperatures storage as the orthodox tolerated levels.

Recalcitrant seeds conservation poses a challenge due to a peculiar characteristic of these seeds that can neither lose large amounts of water nor being stored more than few weeks or months (MARCOS FILHO, 2005). At dispersion moment, these seeds denotes elevated water content, and are metabolically active. Moreover, in several cases these seeds are initiating germination (KERMODE; FICH-SAVAGE, 2002).

Farrant et al. (1985) suggested that there are different types of recalcitrant seeds: minimally, moderately and highly recalcitrant, and that their characteristics are partially related with the habitat. In minimally recalcitrant species, the seeds can withstand to the loss of higher moisture levels and may remain viable for longer periods. These species have a subtropical distribution; therefore, those also tolerate lower temperatures. The *Quercus* sp., *Araucaria hunsteinii* and *Podocarpus henkelii* are example of that.

Moderately recalcitrant seeds might tolerate the loss of moderate water contents and present sensitiveness to inferior temperatures. Several of these species have a tropical distribution, for instance, the *Theobroma cacao* and *Hevea brasiliensis*. In contrast, the highly recalcitrant bears a loss of minimum water content and are not able to survive under lower temperature. These types are usually dwelling in tropical forests, wetlands and mangroves, such as *Avicennia marina*.

In recalcitrant seeds desiccation, several indexes should be considered, such as the safety humidity degree, the critical moisture degree and finally degree of lethal moisture. These indexes varies depending on the selected specie. The safety moisture degree corresponds to a level that could be reached through drying processes without damaging seeds viability (HONG; ELLIS, 1992); the critical humidity degree refers to a value at which the beginning of viability loss is

detected (ANDRADE; CUNHA, 1996). Finally, the lethal moisture degree represents the point at which every seeds loses viability (HONG; ELLIS, 1992).

Drying processes may influence the response of recalcitrant seeds to desiccation (WESLEY-SMITH et al., 2001; JOSÉ et al., 2011). Certain authors argue that rapid seeds' drying process is an effective reducer of damages by viability loss (FARRANT et al., 1985; BERJAK et al., 1990; PRITCHARD, 1991; PAMMENTER et al., 1998). On the other hand, slow drying may promote enhanced desiccation tolerance due to the time, which is responsible to induction and operation of protection mechanisms for seeds. (SILVA et al., 2007).

*Ekebergia capensis* recalcitrant seeds, for example, when rapidly dried maintained viability at a reasonably low water content (0.7/g of water per gram of dry weight); while slow drying led to complete viability loss (PAMMENTER et al., 1998). According to Magistrali et al. (2013), slow drying increased desiccation capacity and storage tolerance in studies with *Genipa americana* seeds. Furthermore, in coffee seeds (*Coffea anefora*), fast drying caused a greater reduction in physiologic quality compared to slow and intermediate drying (ROSA et al., 2005). *Ocotea puberula* specie have their seeds classified as recalcitrant as pointed out by Mori et al. (2012). Therefore, based on what was exposed, the aim of this study was to verify the influence of two drying processes; laboratory stove (fast drying), and silica gel (slow drying) on these seeds quality and determine the critical and lethal moisture degrees.

## 2. MATERIAL AND METHODS

*Ocotea puberula* seeds were collected at Brunópolis, SC, located at latitude 27° 18' 21" S, longitude 50° 52' 06", and altitude of 843m. This city presents an average temperature of 19°C and annual precipitation mean of 1.733mm (GOVERNO DO ESTADO DE SANTA CATARINA, 2013; CLIMATE-DATA, 2014)

The seeds were collected from black-bluish pigmented fruits considered ripe in five matrixes, with the aid of pruning tools. Pulp removal was performed manually with the aid of sieves and running water. The water excess was removed with paper towel. Water content determination was carried out through laboratory stove method, where seeds were submitted to a temperature of 103 °C ± 2°C for 24 hours. This test was performed in duplicate containing ten seeds each

replicate. The outcomes were exposed in percentage, based on seeds moisture weight according to Rules for Testing Seeds (BRASIL, 2009).

For seeds drying, 10 moisture gradients were established starting with 38% (initial humidity's lot), and having 18% as the last gradient, varying from 2 to 2%. The moisture gradients were obtained by weight difference between initial and target weight, according to Hong; Ellis (1996) following formula [1] described below:

[1] Target weight =  $[(100 - U_{\text{initial}}) / (100 - U_{\text{target}})] \times \text{Initial sample weight}$

After each seed had reached the pre-established humidity gradient, the water content was determined by laboratory stove method in order to measure the obtained outcomes with the aforementioned formula.

For slow drying, 11 samples of 160 seeds each were weighed and placed in 11 "mesh type" bags, which allow the passage of air and water. The bags were randomly disposed in a desiccator with silica gel under constant temperature of 25°C. On the other hand, although 11 samples of 160 seeds each were also weighed for fast drying, these were placed in aluminum screens to facilitate water removal. The screens were indiscriminately arranged in a forced ventilation stove with a constant temperature of 35°C. The samples of both drying methods were regularly weighed until reaching the target weight. Then, the samples were conditioned to moisture determination, viability and vigor tests.

Seeds physiological quality was determined by germination tests (BRASIL, 2009); Germination Speed Index (GSI) as suggested by Maguire (1962) formula; Mean Germination Time (T 50) reported by Copeland; McDonald (1985); and Tetrazolium (Tz), regarding Kalil Filho (2008) methodology.

For GSI and T50 germination tests, seeds were conditioned on germitest paper, which were previously moistened with two times the weight of the paper. Then, the species were placed to germinate in a Mangelsdorf germination chamber with temperature of 30°C within a constant light. For each moisture gradient, four repetitions of twenty seeds were utilized, and the integument was manually removed. The results were expressed in regular plants percentage, according to Brazil (2009).

For the tetrazolium test, 60 seeds were divided in four replicates. In addition, those were longitudinally sectioned through embryonic axis center with the aid of a scalpel. Then, these seeds were immersed in the solution of 2,3,5- triphenyltetrazolium chloride (pH 6.5 – 7.0) in the concentration of 0.5% for one hour. Moreover, to support the picturing of every seed detail, two table loupes with magnifying glass were utilized. The criteria analyses were: 1 – viable seed (reddish color), 2- unviable seeds (colorless). Furthermore, the results were expressed in percentage of viable seeds.

The experiments were assembled in a completely randomized design, and data were tested for normality and submitted to variance analysis. The germination data and T 50 expressed as percentages were shown to be non-homogeneous by the Shapiro-Wilk test. Thus, these tests were transformed into  $\arcsin \sqrt{x/100}$ , and the GSI data were converted into  $\sqrt{x + 0,5}$ , according to methodology suggested by Santana; Ranal (2004). The means were compared using Tukey test at 5% probability to prove statistical significance, and the analysis were performed by ASSISTAT® statistical program.

### 3. RESULTS

Seeds submitted to drying methods in both laboratory stove and silica gel took 240 hours (10 days) and 602 hours (25 days) respectively to achieve the last drying point (18%). For this reason, the stove method was classified as fast drying, while the silica gel was classified as slow drying.

The determination of approximated pre-established water levels in this study was possible due to target weight formula (HONG; ELLIS, 1996). The levels were similar to those verified by laboratory stove ascertainment (Table 1).

*Ocotea puberula* seeds germination varied during the drying process. Initial germination speed with newly harvested seeds was 76%, which remained until the moisture of 36% in the slow drying. However, germination potential decreased in fast drying to 62%. It was observed that germination percentage increased with moisture reduction at 34% and 32% in the fast drying decreasing until reaching zero at 22% of water content for both drying types (Graphic 1)

Regarding vigor (GSI and T50), diminutions were observed from 32% moisture levels regardless the

**Table 1** – Drying Hours (HS), gradient preset humidity (GPE) and met (U%) through use of the methodology of the target weight, in seeds of *Ocotea puberula*.

**Tabela 1** – Horas de secagem (HS), gradiente de umidade pré-estabelecido (GPE) e atingidos (U%), por meio da utilização de metodologia do peso alvo, em sementes de *Ocotea puberula*.

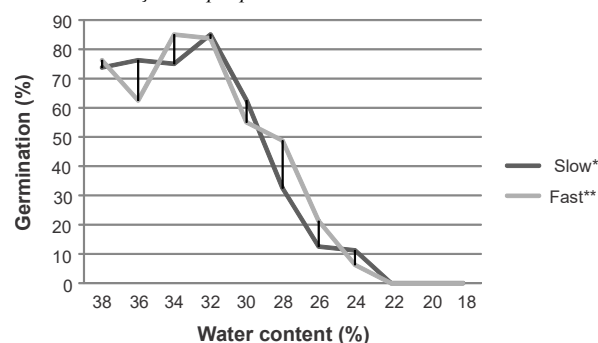
Slow Drying			Fast Drying		
HS	GPE	U%	HS	GPE	U%
0	38	37.79	0	38	38.28
5	36	36.39	2	36	36.34
45	34	33.71	5	34	34.52
98	32	32.13	28	32	32.11
214	30	29.69	50	30	30.37
483	28	29.31	69	28	28.74
483	26	26.63	100	26	26.99
507	24	22.53	148	24	24.77
554	22	23.65	169	22	21.88
577	20	20.79	171	20	20.97
602	18	19.45	240	18	18.99

Source: Elaborated by the author.

Fonte: Produção do próprio autor.

Source: Elaborated by the author.

Fonte: Produção do próprio autor.



**Figure 1** – Germination of *Ocotea puberula*, undergoing slow and fast drying.

\*Linear regression Slow Drying:  $y=20,774+0,212x$  ( $R^2=0,823$ ).

\*\* Linear regression Fast Drying:  $y=20,484+0,218x$  ( $R^2=0,835$ ).

**Figura 1** – Germinação de sementes de *Ocotea puberula*, submetidas à secagem lenta e rápida.

\*Regressão linear Sec. Lenta:  $y=20,774+0,212x$  ( $R^2=0,823$ ).

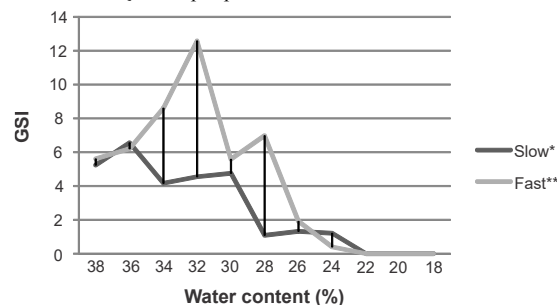
\*\*Regressão linear Sec. Rápida:  $y=20,484+0,218x$  ( $R^2=0,835$ ).

drying type. These indexes decay until reaching zero in the critical humidity degree (Graphic 2 and 3).

The majority of seeds that could not germinate were at a dormant state until 32% of water content. Even though, these seeds imbibed water, there was none radicle protrusion below 32% of water content. Furthermore, the non-germinated seeds were

Source: Elaborated by the author.

Fonte: Produção do próprio autor.



**Figure 2** – Germination Speed Index (GSI) of seeds of *Ocotea puberula*, undergoing slow and fast drying.

\* Linear regression Slow Drying:  $y=12,641+15,332x$  ( $R^2=0,854$ ).

\*\* Linear regression Fast Drying:  $y=13,129+14,338x$  ( $R^2=0,804$ ).

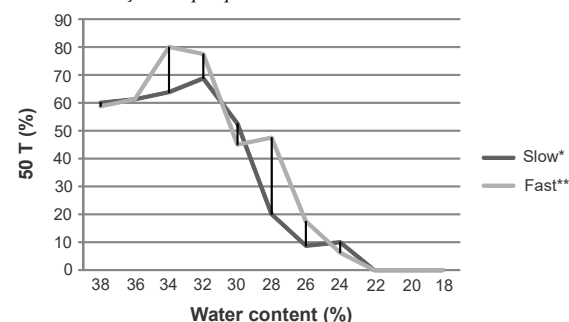
**Figura 2** – Índice de Velocidade de Germinação (IVG) de sementes de *Ocotea puberula*, submetidas à secagem lenta e rápida.

\*Regressão linear Sec. Lenta:  $y=12,641+15,332x$  ( $R^2=0,854$ ).

\*\*Regressão linear Sec. Rápida:  $y=13,129+14,338x$  ( $R^2=0,804$ ).

Source: Elaborated by the author.

Fonte: Produção do próprio autor.



**Figure 3** – 50 T (%) of seeds of *Ocotea puberula*, subjected to slow and fast drying.

\* Linear regression Slow Drying:  $y=20,709+0,253x$  ( $R^2=0,835$ ).

\*\* Linear regression Fast Drying:  $y=20,709+0,229x$  ( $R^2=0,793$ ).

**Figura 3** – T 50 (%) de sementes de *Ocotea puberula*, submetidas a secagem lenta e rápida.

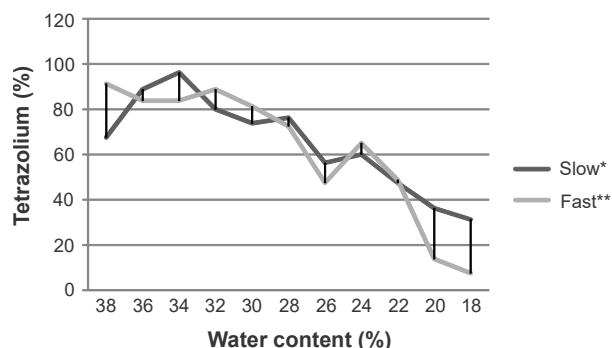
\*Regressão linear Sec. Lenta:  $y=20,709+0,253x$  ( $R^2=0,835$ ).

\*\*Regressão linear Sec. Rápida:  $y=20,709+0,229x$  ( $R^2=0,793$ ).

apparently dead when the conditions were below 32% of water content denoting a dark embryo (data not shown).

Regarding tetrazolium test, more elevated values were verified than those obtained in germination test. For instance, there was none observable seeds germination at 22% of water content, which was considered a lethal level for seeds. However, values superior to 45% were obtained in tetrazolium test (Graphic 4).

Source: Elaborated by the author.  
 Fonte: Produção do próprio autor.



**Figure 4** – Viability (tetrazolium test) of seeds of *Ocotea puberula*, subjected to slow and fast drying.

\* Linear regression Slow Drying:  $y = 17,968 + 0,166x$  ( $R^2 = 0,359$ ).

\*\* Linear regression Fast Drying:  $y = 16,128 + 0,191x$  ( $R^2 = 0,763$ ).

**Figura 4** – Viabilidade (teste de tetrazólio) de sementes de *Ocotea puberula*, submetidas a secagem lenta e rápida.

\*Regressão linear Sec. Lenta:  $y = 17,968 + 0,166x$  ( $R^2 = 0,359$ ).

\*\*Regressão linear Sec. Rápida:  $y = 16,128 + 0,191x$  ( $R^2 = 0,763$ ).

#### 4. DISCUSSION

In accordance with works developed with *Averrhoa carambola* (Oliveira et al., 2011), and *Caesalpinia ferrea* (Gnoatto; Cruz-Silva, 2011) an enhancement in seeds germination when submitted to partial drying were also noticed. It is unclear what factors intensify germination; however, certain studies suggest that normal cellular functions of freshly harvested seeds could be altered when these are dried, which might either deactivate dormancy or accelerate germination processes (BERJAK et al., 1990; PAMMENTER et al., 1998; LEPRINCE et al., 2000).

Critical moisture content found for this specie was 32%, wherein values inferior to this gradient presented decreasing seeds viability until the moisture gradient of 24% reaching, there were observed 15% of germination (slow drying) and 6% of germination (fast drying). From the 22% gradient, there was none germination leading to an interpretation that this gradient is considered the lethal humidity degree. According to Hirano (2002), who worked with the same specie drying point, a sharp decline of *Ocotea puberula* germination initiated when the seeds reached 31.8% of moisture.

Lethals and criticals humidity degrees depend on the specie. In seeds of *Cryptocarya aschersoniana*, from the Lauraceae family, Tonetti (2013) it was concluded

that the critical humidity degree was between 28.1 and 26.3% and the lethal moisture degree was between 21.5 and 18.1%. Nonetheless, for *Archantophoenix alexandrae* seeds with recalcitrant behavior, the critical humidity was 24.84% according to a study performed by Andrade et al (2005).

*Ocotea puberula* seeds germination was statistically different in six points in relation to drying types. However, in general, both methods of drying have showed similar critical and lethal humidity points. Gemaque et al. (2005) found the same outcomes in seeds of *Tabebuia impetiginosa* Mart Standll while Oliva et al. (2012) noticed similar results in seeds of *Crambe abyssinica* Hochst, and Nery (2006), encountered similarities in seeds of *Calophyllum brasiliense* Cambess.

In tetrazolium test, higher viability values were verified than those obtained in the germination test. The studies of Marcos Filho (2005), and Walters et al. (2005) can assist this phenomenon explanation. These researchers affirm that seeds' moisture reduction at intermediate levels of 20 and 33%, range in which the majority of recalcitrant seeds can be stored, maintained considerably elevated respiration rate internally in the seeds. Moreover, the diminution until reach those levels supported the metabolisms active. However, the repair system no longer works perfectly leading to a reduction in germination potentiation of these species. According to Walters et al. (2005), seeds undergo an aging acceleration in this hydration level, which leads those to dead.

#### 5. CONCLUSION

The reduction of water content below 32% harm *Ocotea puberula* seeds germination. Therefore, this value can be considered the critical humidity degree. Moreover, levels of water content inferior to 22% were deliberated as lethal this specie seeds, regardless the drying method (laboratory stove or silica gel).

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