



***Siphoneugena reitzii* D. Legrand: germination, quality and physiological classification of the seeds of a Myrtaceae from the araucaria forest**

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Keywords: Blotter; camboim; recalcitrant seeds; seed storage; substrates.

ABSTRACT – The aim of this study was to describe germination methods, viability characteristics and physiological classification in seed storage of *Siphoneugena reitzii* D. Legrand, a Myrtaceae that occurring in high-altitude areas of the mixed ombrophilous forest. The seeds were collected from five matrices located at “Fazenda das Nascentes” – RPPN Serra da Farofa in the municipality of Urupema, Santa Catarina. The fruits were processed and the seeds exposed for initial characterization of the batches, with determination of water content, germination and vigour and counting and observation of empty or predated seeds. The *S. reitzii* seeds were tested for the best germination conditions on three substrates (sand, blotter and paper roll) and at two temperatures (20 and 25°C). Once the best germination conditions had been found, the seeds were then subjected to physiological classification in storage, carried out by drying them quickly in silica gel until they reached approximately 10% humidity and then germination testing after drying. The results indicated that *S. reitzii* seeds germinate in up to 11 days, with good germination using blotting paper at 20°C (69%) and a moderate rate with sand at 20 and 25°C (55 and 57%). There



were no obvious problems with predated or empty seeds, however, the seeds were classified as recalcitrant and sensitive to desiccation, as they completely lost viability after drying to close to 11% water content. This information allows for better selection of storage methods and seedling production of *S. reitzii*.

***Siphoneugena reitzii* D. Legrand: germinação, qualidade e classificação fisiológica das sementes de uma Myrtaceae da floresta com Araucária**

Palavras-chave: Papel mata-borrão; camboim; sementes recalcitrantes; armazenamento de sementes; substratos.

RESUMO – O objetivo deste trabalho foi descrever os métodos de germinação, as características da viabilidade e a classificação fisiológica em armazenamento de sementes de *Siphoneugena reitzii* D. Legrand, Myrtaceae ocorre em locais de alta altitude da floresta ombrófila mista. As sementes foram coletadas em cinco matrizes localizadas na “Fazenda das Nascentes” – RPPN Serra da Farofa no município de Urupema, Santa Catarina. Os frutos foram beneficiados e as sementes expostas a caracterização inicial dos lotes, com determinação de teor de água, germinação e vigor e contagem e observação de sementes vazias ou predadas. As sementes de *S. reitzii* foram testadas quanto à melhor condição para germinação em três substratos (areia, mata-borrão e rolo de papel) e em duas temperaturas (20 e 25°C). Diante da melhor condição de germinação, as sementes foram então submetidas a classificação fisiológica em armazenamento, realizada com secagem rápida em sílica gel até atingir aproximadamente 10% de umidade e posterior teste de germinação após a secagem. Os resultados indicaram que as sementes de *S. reitzii* germinam em até 11 dias, com boa germinação utilizando papel mata-borrão a 20°C (69%) e uma taxa moderada com areia a 20 e 25°C (55 e 57%). Não foram visualizados problemas evidentes com sementes predadas ou vazias, no entanto, as sementes foram classificadas como recalcitrantes e sensíveis à dessecação, pois perderam totalmente a viabilidade após secagem até próximo a 11% de teor de água. Essas informações permitem a melhor seleção de métodos de armazenamento e produção de mudas de *S. reitzii*.

***Siphoneugena reitzii* D. Legrand: germinación, calidad y clasificación fisiológica de las semillas de una Myrtaceae del bosque de Araucarias**

Palabras clave: Papel de germinación; camboim; semillas recalcitrantes; almacenamiento de semillas; sustratos.

RESUMEN – El objetivo de este estudio fue describir los métodos de germinación, las características de viabilidad y la clasificación fisiológica en el almacenamiento de semillas de *Siphoneugena reitzii* D. Legrand, una Myrtaceae presente en zonas de altura del bosque ombrofilo mixto. Las semillas fueron colectadas en cinco matrices localizadas en la “Fazenda das Nascentes” – RPPN Serra da Farofa en el municipio de Urupema, Santa Catarina. Los frutos fueron procesados y las semillas expuestas para la caracterización inicial de los lotes, con determinación del contenido de agua, germinación y vigor y conteo y observación de semillas vacías o predadas. Las semillas de *S. reitzii* se sometieron a pruebas para determinar las mejores condiciones de germinación en tres sustratos (arena, papel secante y rollo de papel) y a dos temperaturas (20 y 25°C). Una vez encontradas las mejores condiciones de germinación, las semillas se sometieron a una clasificación fisiológica en almacenamiento, realizada secándolas rápidamente en gel de sílice hasta alcanzar aproximadamente un 10% de humedad y, a continuación, a una prueba de germinación tras el secado. Los resultados indicaron que las semillas de *S. reitzii* germinan hasta en 11 días, con una buena germinación utilizando papel secante a 20°C (69%) y una tasa moderada con arena a 20 y 25°C (55 y 57%).

No hubo problemas evidentes con las semillas predadas o vacías, sin embargo, las semillas se clasificaron como recalcitrantes y sensibles a la desecación, ya que perdieron completamente la viabilidad después de secarse hasta cerca del 11% de contenido de agua. Esta información permite seleccionar mejor los métodos de almacenamiento y la producción de plántulas de *S. reitzii*.

Introduction

In the production of native tree seeds, it is important to determine some of the essential characteristics for propagation, likewise germination, vigor and storage potential [1]. In this sense, environmental factors are essential and have a direct influence on germination potential, in which the availability of water, temperature, oxygen and the type of substrate are considered to be the most important external factors linked to quality, viability and dormancy [2][3]. Likewise, determining the physiological behavior of seeds during storage is important for forestry, as it provides useful data for making decisions about when to sow seeds in nurseries or direct plantings, avoiding the unnecessary loss of viability and vigor of the propagules [4].

In this context, Myrtaceae is a family of plants found in tropical and subtropical environments, which plays a significant role in the natural regeneration of ecosystems [5]. The dispersal and propagation of this group is carried out primarily by seeds, and it is essential to know the characteristics of the propagules both for the conservation of biodiversity and for reforestation initiatives [6][7].

Among Myrtaceae with scarce information on their reproductive and silvicultural behavior, *Siphoneugena reitzii* D. Legrand, popularly known as camboim, is a specie with a tree-like habit, heliophytic, early secondary/late secondary that can reach a height of up to 15 m. The bark is smooth with a greyish or yellowish coriaceous lamina [8][9]. It occurs in high-altitude areas of southern and southeastern Brazil, such as the dense ombrophilous forest and the high-montane mixed ombrophilous forest [10] and has been classified by Gomes [11] as a “relic species,” as it thrives in high-altitude areas above 1,800 m.

The species produces very hard and heavy wood, locally used for fence posts or as firewood [8] and despite the apparent uniqueness of the establishment of this species, *S. reitzii* have been gaining attention due to their potential for use in the food, cosmetics and pharmaceutical industries

[11] due to the presence of volatile oils with anti-inflammatory activity [12] and antimicrobial action [13]. In addition to their ecological importance in restoration, they act as bioindicator plants for dystrophic soils with high acidity [11].

Despite the proven potential of this species, due to its specific occurrence, little is known about its silvicultural characteristics. It is known that its fruits are small circular glabrous berries, reddish in color when immature and purple when ripe, which contain 1 to 2 seeds with eugenoid embryos [10] that are usually part of the seed bank in mixed ombrophilous forests at high altitudes [14][15]. However, there are no conclusive studies on germination, seed quality and physiological classification for *S. reitzii*. In this way, the aim of this study was to describe germination in different substrates and temperatures, quality and physiological classification of *Siphoneugena reitzii* seeds.

Material and Methods

Collection area

The study was carried out using *S. reitzii* seeds obtained from five matrices located at the “Fazenda das Nascentes – RPPN Serra da Farofa – owned by the Klabin SA company, located on the border between the municipalities of Paineal and Urupema, Santa Catarina, Brazil, at an altitude of 1,425 m and with a Cfb climate (humid subtropical mesothermal), according to the Köppen classification [16][17]. The region’s climatic conditions include annual temperatures of around 12 to 14°C, with mild summers, no dry season, recurrent severe frosts and well-distributed rainfall throughout the year, ranging from 1,600 to 1,900 mm per year. Snow is also common in the coldest months [17][16]. The phytoecological region is classified as araucaria forest (mixed ombrophilous forest) subclassification highmontane [17].

The trees were selected at a minimum distance of 200 m apart, in order to avoid obtaining parents with genetic similarities. Initially, the ripe fruits (dark

purple in color) were collected using a lopper and then taken to the Forest Seed Laboratory (LASF) at the “Centro de Ciências Agroveterinárias (CAV)” of the “Universidade do Estado de Santa Catarina (UDESC)”, where they were pulped under running water. The seeds remained distributed on trays with paper towels for around 8 hours to dry on the surface and were then subjected to characterization and the other tests. It should be noted that the seeds from the matrices were analyzed as a single batch.

Initial characterization and testing of temperatures and substrates

For the initial characterization of the lots, germination, vigor and water content tests were carried out. The water content was determined according to the Rules for Seed Analysis [18], with the samples in an oven at $105 \pm 3^\circ\text{C}$ for 24 hours with two repetitions of 3 g per repetition. The seeds were weighed on an analytical balance in aluminum capsules with a determined weight and placed in an oven. The water content was calculated based on the weight of the wet seeds. To assess predated and empty seeds, 100 seeds of each species were cut lengthways with a scalpel and analyzed under a stereomicroscope.

S. reitzii seeds were exposed to different treatments to assess the influence of different substrates and temperatures on seed germination. The germination tests were carried out in a 2 x 3 factorial design (temperature x substrate) in a B.O.D. chamber. The seeds were placed to germinate in three substrates: in acrylic gerboxes with autoclaved sand (SA), blotting paper (mt) and paper rolls (RP) moistened to 2.5 (mL) times the weight of the paper (G), and at temperatures of 20 and 25°C in constant light [18][19]. Evaluations were carried out daily from the 4th day until the 40th day of the test. After the end of the experiment, the ungerminated seeds were opened with a scalpel to check if they had rotted.

Four replicates of 25 seeds were used per batch/treatment, where the percentages of normal, abnormal, dead and hard seedlings were assessed, in accordance with the Instructions for Seed Analysis of Forest Species [20], and the seeds were considered germinated when they had more than 2 mm of primary root. At the same time, the germination percentage index (G%) [18], the germination speed index (GVI) [21] and the mean germination time (MGT) [22] were evaluated.

Physiological classification of *Siphoneugena reitzii* seeds

For the physiological classification of *S. reitzii*, the protocol proposed by Hong and Ellis [23] and adapted by Sacandé et al. [24] was used. The first stage, before drying, a seed germination test carried out at 20°C on blotting paper. On the second stage, the rapid drying method was carried out by placing the seeds in airtight recipients exposed to silica gel at a temperature of 23°C , until they reached approximately 10% humidity. The weight was checked on an analytical balance every hour for the first 8 to 124 hours, and every 12 hours thereafter. The loss of water from the seeds was monitored using the mass difference calculation proposed by Sacandé et al [24].

In the third stage, the seeds that reached low water contents were stored in a freezer set at -20°C for three months in airtight plastic bags. All stages were tested for viability using a germination test in a B.O.D. set at 20°C for *S. reitzii*, both on blotting paper, according to the results of the previous tests. Based on the germination rate obtained, the seeds were classified as orthodox, intermediate or recalcitrant.

Data analysis

The germination and seed physiology tests were analyzed using the R statistical program with the “ExpDesp” package [25], which tested the normality and homogeneity of the data obtained in each of the analysis stages. The Entirely Randomized Design (ICD) was used for the germination analysis. After obtaining the data, normality was assessed using the Shapiro-Wilk test. Analyses of variance were carried out and mean tests were conducted (Tukey at 5% probability). Given the assumptions of normality for physiological quality in storage and germination, the data was submitted to analysis of variance and the means to the t-test at 5% probability.

Results

Initial characterization and testing of temperatures and substrates

The moisture content obtained for the *S. reitzii* seeds was 34.2%. It should be noted that all the seeds evaluated showed no apparent predation, damage, parasites or empty propagules and all the germinated seeds formed normal seedlings, while the non-germinated seeds showed rot (Figure 1).

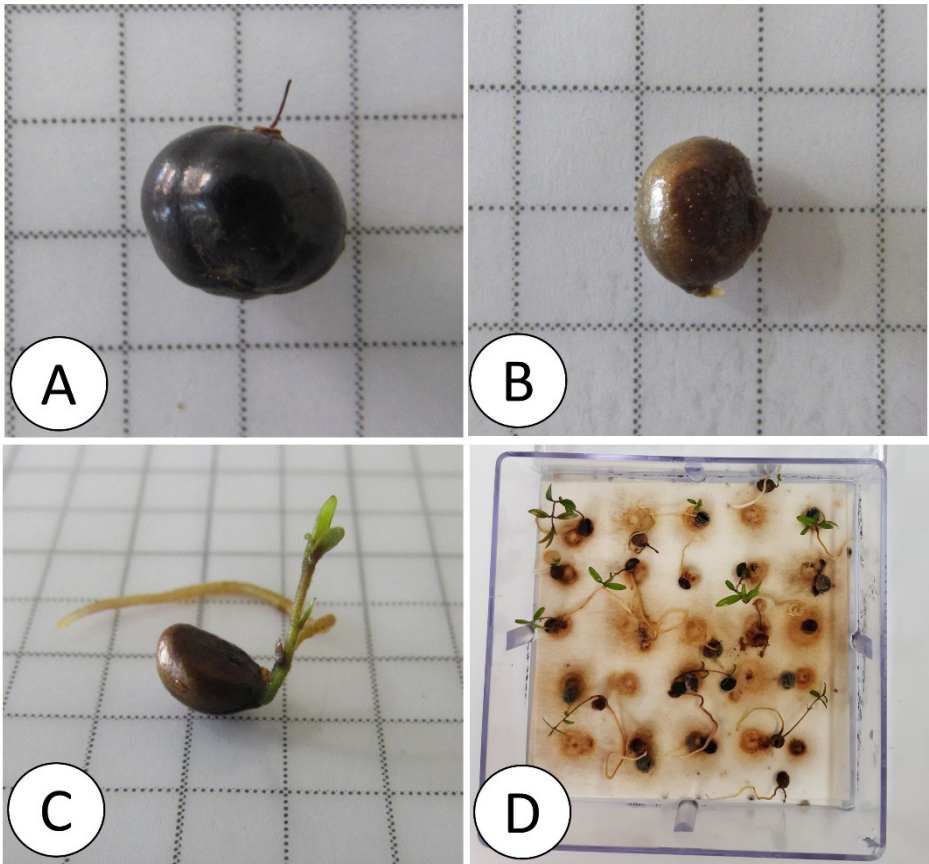


Figure 1 – *Siphoneugena reitzii*. A = Mature fruits in pre-processing. B = Seed. C = Seed in the process of germination. D = Seeds germinated in a blotter substrate at a temperature of 20°C.

The test with *S. reitzii* seeds showed an interaction between germination and the substrates tested. The highest average germination occurred on blotting paper at 20°C, followed by sand at 20 and 25°C and blotting paper at 25°C (Table 1; Figure 1D).

Table 1 – Germination (G%), germination speed index (GVI) and mean germination time (MGT) of *Siphoneugena reitzii* seeds, at different temperatures and substrates.

Substrates	Temperature (°C)					
	20	25	20	25	20	25
	G%		GVI		MGT	
Sand	55 ab	57 ab	0.37	0.37	11	11
Blotting paper	69 a	45 abc	0.33	0.21	11	11
Paper rolls	27 bc	17 c	0.23	0.18	13	12

Averages followed by the same letter do not differ statistically by the Tukey test at 5%. According to the Levene test at 5% significance, the variances can be considered homogeneous.

The treatments using roll paper rolls did not show satisfactory germination results (Table1). The MGT showed that germination occurs in 11 days, while the germination speed index (GVI) was not significant for all treatments.

Physiological classification

S. reitzii seeds had a water content of 34.2% and a germination percentage of up to 69% before

drying. After drying (to approximately 11% humidity in 4 days) the seeds completely lost their viability, as indicated by the germination test (Tab. 2).

Table 2 – Germination and water content of *Siphoneugena reitzii* seeds before and after drying, following the stages of Hong and Ellis protocol.

Hong and Ellis protocol*	Seeds condition	Water content (%)	Germination (%)
Stage 1	Before drying	34.2	69
Stage 2	After drying	11	0
Stage 3	Freeezing at -20	NA**	NA

* Hong and Ellis [23] protocol adapted by Sacandé [24]. ** Not aplicable.

Since the seeds do not germinate, they lost viability after drying and were not subjected to freezing according to the protocol.

Discussion

The satisfactory germination percentage of *S. reitzii* reinforces the fact that its seeds germinate quickly, with no major impediments to radicle emission, such as dormancy or low physiological quality, in a pattern similar to that observed in *Myrceugenia euosma* [26]. Its water content is similar to that obtained by other Myrtaceae, such as *Eugenia rostrifolia* D. Legrand (34%) and *Campomanesia xanthocarpa* (Mart.) O.Berg (33%) [27]. It should also be noted that the humidity found for its seeds is a common characteristic in other Myrtaceae [19].

The temperature of 20°C favored a higher germination percentage when blotting paper was used, indicating a possible relationship with the availability of moisture provided by this substrate, which also contributed to higher germination in *Blepharocalyx salicifolius* (Kunth) O.Berg (Myrtaceae) seeds [28]. The use of sand had no statistically significant effect on the germination percentage at 20 and 25°C. This suggests that sand may have varied humidity between treatments due to its drainage capacity, which is unsuitable for fast-germinating seeds, as observed in other Myrtaceae species [29] [19][30][31].

On the other hand, the use of paper rolls is not recommended for this species due to the low germination values observed, possibly due to the

low light intensity and the availability of moisture for the seeds, which also affected the germination of *Eugenia umbellata* Spreng. seeds [29]. In addition, at a temperature of 25°C, a decrease in germination was observed compared to 20°C, perhaps related to the physiological characteristics of the seeds in the face of the exposed temperature. This species is native to places with high altitudes and low temperatures [10]. In fact, the municipality of Urupema, Santa Catarina State, where the seeds were collected, has average temperatures that oscillate around 18°C during the summer, when the ripe fruits were collected. Therefore, it can be inferred that the ideal temperature for the germination of *S. reitzii* is related to its region of origin, as observed for *Eugenia involucrata* [19].

The ability to germinate at temperatures close to local averages, the quality of the seeds and the adaptation to different types of substrates suggest an ecological plasticity that can be exploited in conservation and propagation programs for the species [26]. Furthermore, understanding the optimum germination conditions can facilitate the production of seedlings and expand the cultivation of *S. reitzii* in nurseries, contributing to initiatives on its conservation and sustainable use [1][2].

The low tolerance of *S. reitzii* seeds to desiccation at 10-12% moisture allows them to be classified as recalcitrant according to their physiological performance in storage, and Myrtaceae often includes several recalcitrant species [32] [33]. The causes of desiccation intolerance may be related to several factors, including the accumulation of insoluble reserves, cellular dedifferentiation, the efficiency of the antioxidant system and the accumulation of

protective proteins and oligosaccharides, as well as a disorder in membrane repair metabolism [34][32]. This initial parameter can inform future research into the critical and lethal degree of humidity and points to the need for specific precautions when storing these seeds.

Conclusion

Siphoneugena reitzii seeds showed a response to the substrates and temperatures tested, with the highest germination on blotting paper at 20°C, followed by moderate germination in sand at 20 and 25°C. The seeds germinate quickly, have no apparent problems with empty seeds or predation and form normal seedlings, demonstrating the viability and potential of this species for propagation. However, the seeds are recalcitrant, losing their viability completely during desiccation at approximately 10% humidity.

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