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GERMINATION OF *HYMENAEA COURBARIL*: STORAGE BEHAVIOR, ORIGIN, AND SUBSTRATE

***REGNIER, Leonardo de Lima Pereira**

Master's student - Department of Botany, Institute of Biosciences University of São Paulo (USP), Brazil

ABSTRACT

Hymenaea courbaril is an endangered species, promising to reforestation programs and mainly explored as a wood source. The available information concerning long-term storage methods, seed recalcitrance, parental, and substrate influence is scarce. This study focused on the seed behavior according to population origin and during one-year storage, also testing the efficiency of the low-temperature conservation. Variations between the uncertainty indexes were found to the studied populations after long-term storage. There was no significant loss of the germination potential in consequence of the prolonged storage period. Although, older seeds promoted gradually greater delayed germination. Germination speed, synchrony, and uncertainty indexes were substantially different between the -20º conservation and control. *H.* courbaril seeds are capable of long-term storage without losing their germination potential, indicating an orthodox behavior.

Keywords: Forestry seeds, Fabaceae, Seedling production, Seed bank

INTRODUCTION

Seed deterioration is the main problem in production systems [1]. Understanding deterioration and seed lots aging process leads to techniques to minimize the rate and impact of the degradation process, as well as the negative impact of suboptimal seed and seedling production [2].

Orthodox seeds are capable to remaining viable after desiccation and are capable to being preserved at low temperatures, being possible to long-term storage in germplasm banks [3]. In contrast to orthodox, recalcitrant seeds lose their germination potential when they reach low moisture content [3]. Therefore, studies concerning seed maintenance are a central facet of biodiversity conservation, to avoid species extinction, through germplasm bank conservation methods [3].

Hymenaea courbaril L., popularly known as "Jatobá", is a deciduous tree, with adult individuals reaching 20 meters long [4]. Presents a wide geographic distribution, from southern Mexico to southeast Brazil [5]. It has a high tolerance to great climatic and hydric variations and also low nutritional requirements [6], [7]. Moreover, this species belongs a climax ecologic role [8], with a rare occurrence, sparse distribution, frequently found in dryland rainforest, and sometimes in high floodplains [7].

Hymenaea courbaril seed behavior is uncertain since there are restricted investigations on the effects

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of long-term exposure to sub-zero temperature, or even desiccation, influence over the viability of this species' seeds [9]. Some authors indicate that they are possibly intermediate or orthodox [5], but appropriate investigations are still required.

Beyond the ecological relevance of *H. courbaril*, this species has an important economic role. Its individuals are very productive [7], and fruits are very important to local communities, used in traditional medicine and culinary. Wood is very important, and is the main explored product, being a very valuable resource in the Brazilian and also international markets [7], [10]. The deforestation of this species natural habitat, and commercial exploration as a timber source has led this species to extinction [11], [12].

The main hindrance found in native seedling production is the general pattern of slow development, especially for ecologic climax species [13]. Due to the long-term production of these species, the simpler techniques have not been able to provide adequate production to fulfill the reforestation program's demand [13]–[16]. Propitiating the development of techniques that generate higher quality seedlings and/or reduce the time of the production process [13]. It has been shown that *H. courbaril* has potential for soil recovery from degraded areas [17], and even to promotes canopy formation [18]. The benefits of this particular species in the seedling production context process are the potential for quick production and high seedling survival in the field [4], [19]. These features favor faster seedling production and establishment in the field, which also promotes the regular use of this species in forestry and afforestation programs [19].

Parent-plant origin is an important aspect to evaluate because it could affect the germination, dormancy processes, and seedling vigor [20]. Some authors have already pointed out some differences according to parent plant origin, although these are features not completely understood [20]. This kind of knowledge propitiates genetic enhancement and promotes regeneration programs, silviculture, and conservation more effectively [20].

The enhancement of production techniques is relevant, especially due to the recent rise in environmental destruction. Thus, recovery programs promote a continuous demand for forest recomposition [11], requiring the development of better practices to promote quicker and uniform germination and also better seedling establishment [21], [22]. In this scenario, native species are drawing attention because of the information gap concerning the germination aspects of those species [23]. Since the recognition of these aspects could also be applied to understand the ecologic dynamics in a natural environment.

Thereby, this study aimed to uderstand seed behavior according to population origin, substrate, and during one-year storage at -20 °C. Trying to understand how these aspects could influence the principal germination parameters and evaluate their importance in the seedling production context.

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MATERIAL AND METHODS

This study was conducted in the city of Cotia (23°36'30.0"S 46°50'48.9"W), which started at the end of 2018 until the end of 2019. The region presents the Cwa climate [24].

Plant material was harvested on 31 October 2018, from two populations, 20 km away from each other, and used to measure variations associated with seed origin. Only mature fruits were gathered from the ground. Seeds were removed from the fruits, and the endocarp was grated manually with a blunt knife. Seeds were planted in sterile thin quartz sand (1 mm particles). The assessment of the factors time, population, and treatments was organized as a randomized block design (RBD), as shown in Table 1.

Table 1: Randomized block design of experiment, with population and treatment associations.

Storage treatment / Origin	-20 °C	Control
Population I	150 (3x50)	150 (3x50)
Population II	150 (3x50)	150 (3x50)
Total	**300**	**300**

The experiment was always performed into three repetitions with 50 seeds for each treatment. The block design was repeated after three storage periods, 0, 180, and 365 days of treatment exposure. Seeds were kept in colorless polypropylene boxes. The seed weight was measured before seed sowing. During the analysis period, each box received a pulverized 500 mL water supply twice a day at 8 am and 5 pm. Emergence was recorded for 75 days.

Substrate influence was measured by comparing the control of the population II pattern at the first execution of the experiment, with the germination obtained to 400 seeds also from population II, but sowed in 280 cm^3 greenhouse tubes, with a prepared organic-based substrate composed of 2 liters of local soil, 8 liters of rice husk, 5 liters of Basaplant® forestry substrate, 46.5 g of Yoorin K® potassium thermosetting fertilizer, and 46.5 g of gradual release fertilizer Osmocote Plus®.

The analysis of the germination pattern was conducted through scatter plots, using the local regression (LOESS) method to estimate the mean line and confidence interval of 95%, using the package ggplot2 [25] with stat_smooth function. Statistical tests concerning the most common germination indexes were performed using R software [26], the GerminaR calculator [27], [28]. More detailed information about the tool is available in Lozlano-isla et al. [27]. The results were tested by ANOVA and in sequence submitted to the Tukey test with a critical p-value of 5% (α <0.05).

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RESULTS AND DISCUSSION

Seed origin

The germination pattern was consistently different between the populations (Figure 1). It is possible to notice that the storage time provides a gradual rise in the uncertainty, as demonstrated by the greater confidence interval, especially for population II. What also generates a significant variation in the uncertainty index obtained during the last analysis period (Figure 2). The germination proportion and seed origin are important aspects to be considered in a seedling production context. In general, a parent population is chosen as the main source of seeds to produce new seedlings.

We obtained significant differences between the analyzed populations. At any studied period, we observed a better germination proportion to population II, according to the local regression method (Figure 1). However, the obtained indices of germination proportion or mean germination time did not present variations comparing the populations.

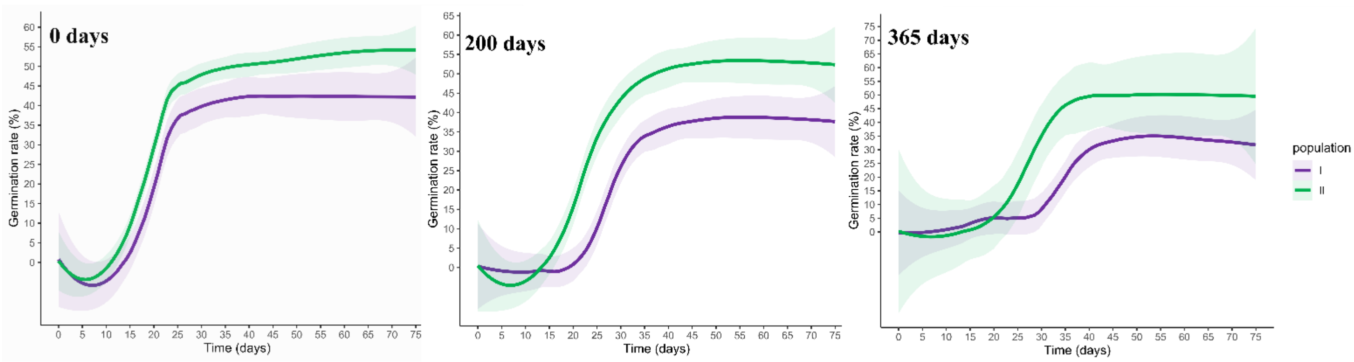

Figure 1: Germination pattern according to seed origin: Population I (■) and Population II (■), and also its storage time. Lines were drawn using the local regression formula, and the shadow area represents the confidence interval of 95%.

It is possible to notice that, in general, seeds from different parent populations had variations in germination patterns (Figure 1). Especially for the first and second execution of the experiment, in which the confidence interval does not overlap with each other, indicating that, to the local regression approach (loess), there is a significant difference between those periods, until the end of the period analysis when the confidence interval starts to overlay each other. However, in the third execution of the experiment, the confidence interval of both populations expanded and their projections touched each other almost all the studied period, indicating that the observed differences are not so clear.

Variations in seed vigor according to the origin were previously observed. Parent plant height is correlated with seed weight in some families, Fabaceae is one of them [29]. Although, to *H. courbaril* previous studies differences in germination speed and proportion were not found to parent plants that were 30 meters away from each other [20]. Our study also corroborates that there are no substantial variations according to the parent plants since differences were

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not observed in those indexes. It is important to note that the distance between the selected populations is beyond the estimated gene flow limit of 7.123 m [30].

It is recognized that *H. courbaril* presents an allogamous reproduction system [30]. This promotes higher variations inside the population but reduces genetic divergence when populations are compared. What also directly impacts the studies of those species and increases the importance of adequate sampling. In the bushlands, *H. courbaril* has a low occurrence, which is less than 1 individual/hectare, and also an irregular distribution [7]. Therefore, the present study data emphasize that even greater distances than 20 km, it still not possible to observe major differences in most of the germination indexes.

The uncertainty index is an adaptation of the Shannon diversity index [27]. Hence, lower values are obtained for more homogeneous germination patterns (Figure 2). The other analyzed germination indeces did not present statistical significance.

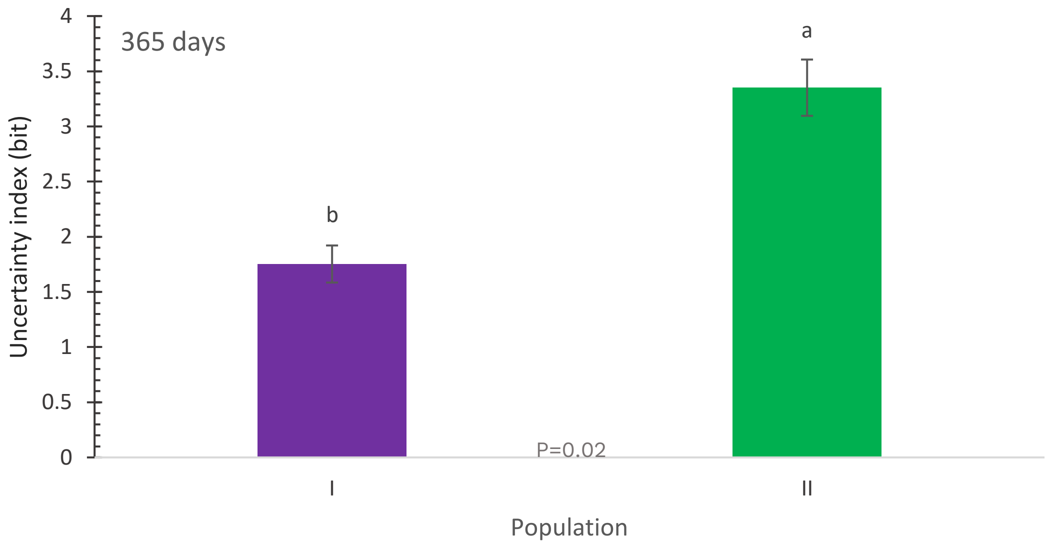

Figure 2: Uncertainty index between the populations after 365 days of storage. Different lowercase letters indicate statistical significance when α ≤5%. Error bars present the standard error mean.

Previous variations in the uncertainty index were also observed for *H. courbaril* according to the harvesting method [31]. Several environmental conditions could influence the process of seed soaking [32]. Oliveira & Aloufa [33] mention that the number and distribution of pores, water availability, temperature, hydrostatic pressure, and surface area are some of the conditions that influence the time required to start the germination process. Because of the greater storage time, the water content could vary between seeds from the same seed lot, and these factors could drastically influence the uncertainty of germination.

Seed storage behavior

Adopting the local regression method, the variations in the germination pattern were only significant during the exponential stage (Figure 3). The time of storage had not impacted the germination potential. Overall, we observed a slight

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reduction in the germination proportion observed in population I, while the proportions were very similar to population II.

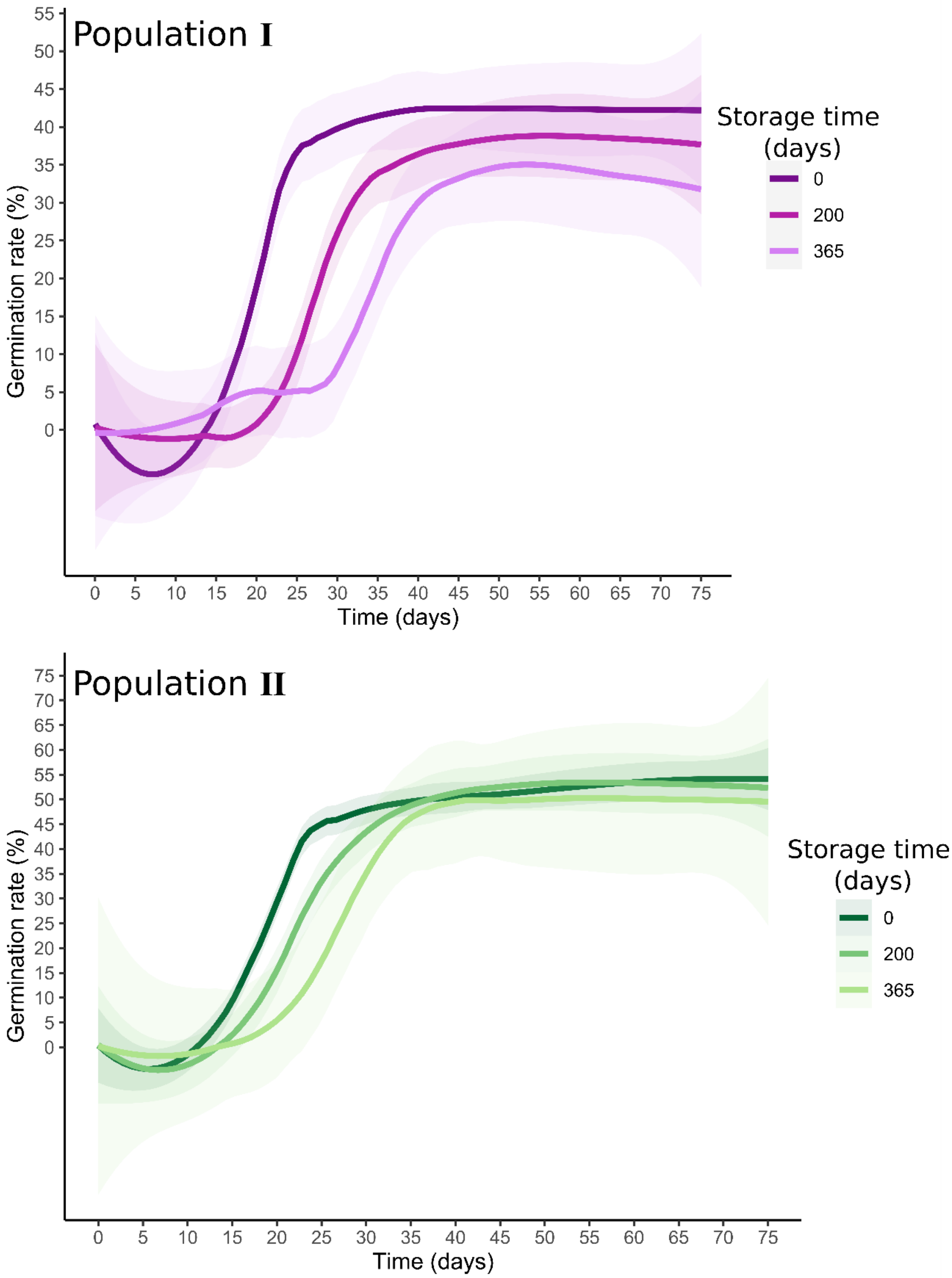

Figure 3: Storage time influences the germination pattern of population I and population II. Lines were drawn using the local regression formula, and the shadow area represents the confidence interval of 95%.

H. courbaril storage behavior has not been extensively studied. Also, previous authors mentioned that intermediate or orthodox are possible classifications to the seeds. Our results indicate that this species could

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be successfully stored for one year without a substantial reduction in the germination potential of the seeds. It is important to note that the time required for the seeds to reach the exponential phase was gradually delayed (Figure 3). Previous studies indicate that main seedling emergencies occur in concentered time, starting about the 8th until the 16th day after sowing [34]. This information is attested to the first execution of the experiment. Withal, to both populations, after 200 and 365 of storage, this period was slightly delayed, notwithstanding the similar germination proportions at the end of the study period were obtained. The analysis of the germination indexes did not provide significant variations between the first and subsequent executions of the experiment. Although, the synchrony index has consistent discrepancies according to the storage time (0, 200, and 365 days) to population I (Figure 4).

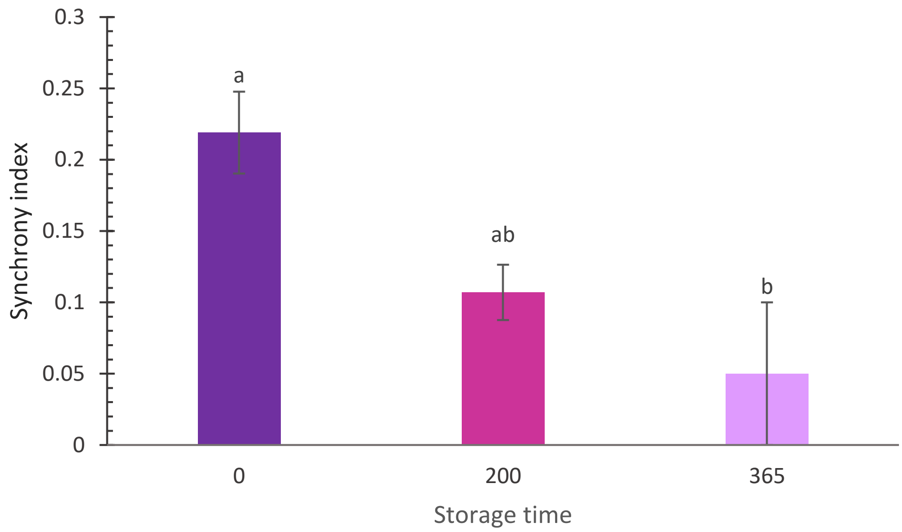

Figure 4: Synchrony index variation for population I according to storage time. Error bars present the standard error mean. Different lowercase letters present significant variations, adopting α ≤5%.

Reduced synchrony is very important in the ecologic context, large seed sets that present reduced synchrony could play the role of a seed bank, continuously or during a prolonged period. Hence, they are capable to keep the recruitment [35]. It is notable that greater time also propitiates reduced synchrony (Figure 4). This favors a long duration of seedbanks from a single cohort. Thus, even one year after the seed formation had passed, those seeds were still capable of germinating and were less likely to germinate concomitantly with each other.

It is expected that a greater time of storage generate less uniformity to germinations. During the time of storage, it is expected a reduction in the water content of the seeds. Orthodox or intermediate seeds are resistant to this natural drought process [3]. While the recalcitrant seeds are very susceptible to modifications of moisture content, reducing seed viability. When seeds are exposed to favorable germination conditions, embryo development is retaken after the imbibition process [21], [36]. It was still unknown the *H. courbaril* behavior [3], [5], but our data suggest that this species´ seeds are capable of long-term storage without losing their germination potential, even after 200 or 365 days after harvest. Hence, the obtained data indicate the orthodox behavior of the seeds.

As a whole, the germination rate of *H. courbaril* is variable according to the study. Some authors attest that the germination rate reaches approximately 81 %, a satisfactory value, at 80 days after sowing [34]. Other authors indicate a wider range of germination rates, from 89.5% [37] to 60% [38]. Recent studies, using similar conditions [31], presented comparable values of germination rate, around 50%. It is expected to find variations in seedling emergence and germination potential between seed lots since these species are continuously submitted to the evolutionary process of the natural environment [30], [36]. Producing regional limitations of some study findings [30].

Seed conservation

For both analyzed periods, the storage at -20ºC seems to be efficient, reducing seed lot deterioration (Figure 5). We obtained a similar germination rate to the previous executions of the experiment when the seeds were maintained at low temperatures. While seeds that are stored at room temperature had a gradual reduction in germination proportion and expanded confidence interval. At 365 days of storage, there were significant discrepancies between the germination patterns of the treatments to most of the germination trajectory (Figure 5), since the confidence intervals do not overlap each other, indicating substantial discrepancies between the treatments adopting the local regression method.

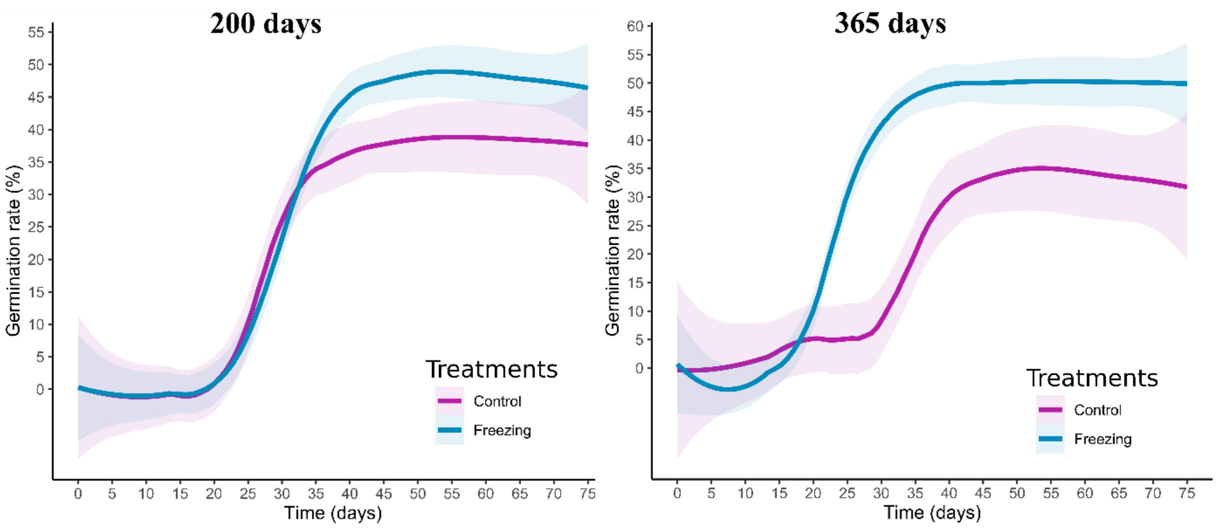

Figure 5: Seed storage at -20°C (freezing treatment) and seeds kept at room temperature (control treatment) germination pattern. Lines were drawn using the local regression formula, and the shadow area represents the confidence interval of 95%.

Low-temperature treatments are usually to keep seed viability during greater storage times. In the experiment, there was significant conservation of the seeds at the last execution. Thus, it seems to be an important conservation method to keep seeds at low temperatures if it is expected to one year or greater, storage. Until 200 days of storage, it seems that it is the beginning of the differentiation of the germination

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patterns (Figure 5). Although, at the end of the analysis, the confidence interval starts to overlap with each other.

There were consistent variations between the germination speed, synchrony, and uncertainty indexes (Figure 6). In general, preservation under low temperature keeps the germination speed and the uncertainty of germination unchanged from the first executions of the experiment. However, the synchrony index was reduced regardless of the treatment. Thus, the loss of synchrony on the germination patterns seems to be related to the seed aging process.

Germination indexes are important to physiologists, seed technologists, and also for ecologists because it is possible to predict the species capacity of their harvest seed to spread germination through time [39]. From this perspective, the synchrony on the germination process is very important since homogeneous germination requires less time to keep a seed lot in the greenhouse or BOD chamber [31]. Posterior seed development stages and further production steps could be done in sequence since germination occurs at concentered periods [32]. While from an ecology perspective, sparse germination patterns lead to the long-term duration of a seed bank.

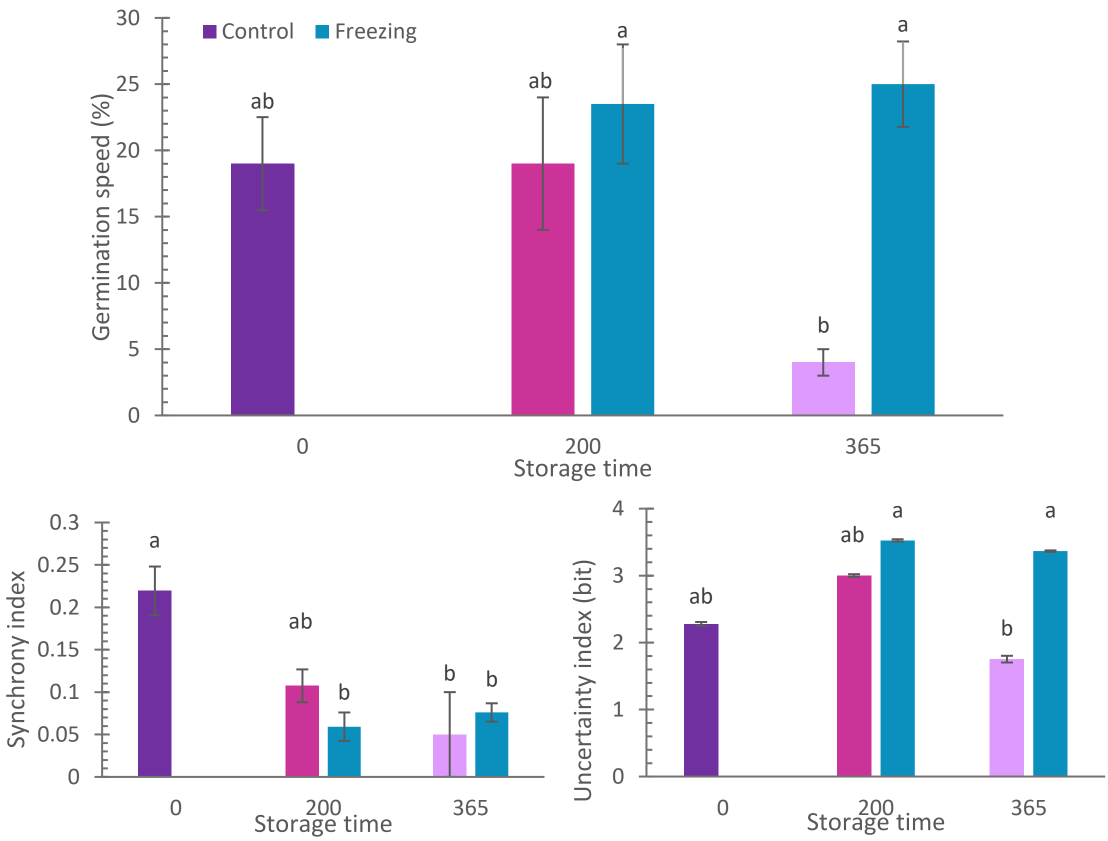

Figure 6: Germination speed, synchrony, and uncertainty index according to storage time, and method: Freezing at -20ºC (■), and control (storage at room temperature) to population I. Different lowercase letters present significant variations adopting α ≤5%. Error bars present the standard error mean.

In a seedling production context, it is preferable to keep seeds at room temperature, since cryo conservation is very expensive. Thus, this kind of conservation approach is only considered when is strongly

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recommended. Some authors declare that *H. courbaril* seeds could be preserved for at least two years [6]. Previous studies concerning the best cryopreservation method were conducted to evaluate possible temperatures to maintain *H. courbaril* seeds [3]. The authors concluded that temperatures between the range of -30 and -175 ºC are possible alternatives to this seed conservation. Our study brings new findings that -20 ºC is a possible temperature to keep seeds for long-term storage, but only significant differences are observed after approximately one year of storage. Thereby, only if the producers are intended to store seeds for more than 200 days, the freezing option seems to be more profitable.

Substrate influence

Only slight differences between the germination could be observed between the studied substrates (Figure 7). The local regression indicates a clear difference between those treatments during a short window between the 20 and 25th day, but after that, both germination patterns overlap each other and the germination proportion is almost the same.

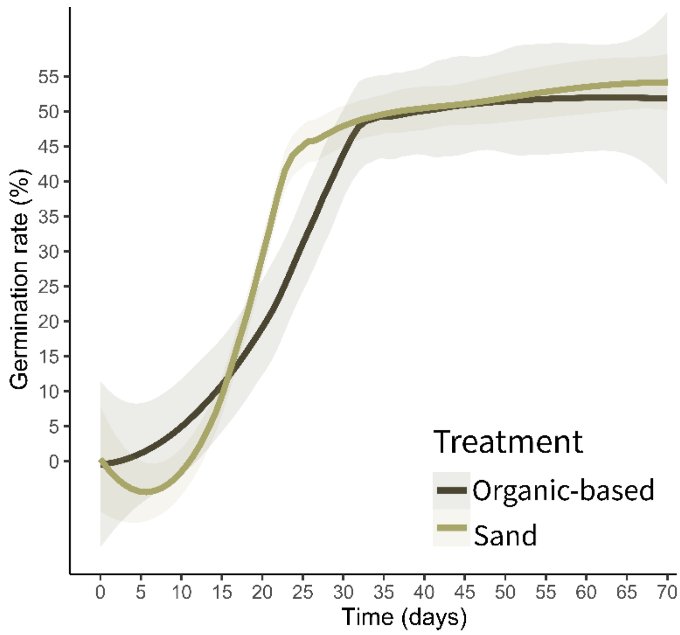

Figure 7: Germination pattern according to the substrate used: sand (■) or organic-based (■).Lines were drawn using the local regression formula, and the shadow area represents the confidence interval of 95%.

The substrate also influenced the germination proportion. Pioneer evaluations concerning the substrate influence on *H. courbaril* germination were executed, and the hummus (another organic-based substrate) was effective, promoting slightly better germination indexes [11]. However, the germination proportion values were not statistically discrepant. After that, initial evaluations using sand at high temperatures indicated that this substrate was adequate to propitiate better germination rates [9]. Pagliarini et al. [40] have mentioned that sand- or commercial organic-based substrates are both acceptable to this species germination, even though these methods presented variations in the germination speed index. The present study data contributes to the previous information, that there are no substantial discrepancies between those methods. Although, we did not find variations in any of the germination indexes.

Some of these variations were explored by Costa et al. [19], which measured the influence of shading and different proportions of organic and vermiculite substrates. The mentioned authors found that shaded environments associated with greater proportions of cattle manure (60-70%) and lower proportions of vermiculite (40–30%) could result in faster germinations. While in brighter environments, the opposite proportions promoted faster germinations. Our observations conflict with this information, as relevant variations in germination speed were not observed.

CONCLUSION

There were no significant differences between the studied indexes according to population origin. A gradual rise in the storage time leads to reduced synchrony and greater uncertainty, but there is no significant loss of germination potential. *H. courbaril* seeds are capable of long-term storage without losing their germination potential, indicating orthodox behavior. Conserving seeds at -20 ºC decreased seed deterioration after long-term storage. However, only if the producers are intended to store seeds for more than 200 days, the freezing option seems to be more profitable.

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