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EFFECTS OF PRE-GERMINATION TREATMENTS AND STORAGE ON GERMINATION OF *Astronium fraxinifolium* SCHOTT (ANACARDIACEAE) DIASPORES

EFEITOS DE TRATAMENTOS PRÉ-GERMINATIVOS E DO ARMAZENAMENTO NA GERMINAÇÃO DE DIÁSPOROS DE *Astronium fraxinifolium* SCHOTT (ANACARDIACEAE)

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ABSTRACT

The goal of this study was to evaluate the germination and the storage capacity of *Astronium fraxinifolium* diaspores. Six pre-germination treatments were used in the experiment: control treatment (intact diaspores); diaspores immersed in water at room temperature (25º C) for 5 min; diaspores immersed in water at 70° C for 5 min; diaspores immersed in water at 100° C for 5 min; diaspores immersed in sodium hypochlorite solution (1:1000) for 2 min; and diaspores mechanically scarified with sandpaper #80. To evaluate storage conditions, we tested two different types of packaging (permeable paper bag and transparent glass jar) and two environmental conditions (cold chamber and room conditions), resulting in four treatments. The germination tests were performed for zero (control) and 60, 120, 180, 240, 300 and 360 days after storage. The effects of different treatments on germination and storage of diaspores were evaluated by ANOVA, followed by Tukey test. Regarding to pre-germination treatments, high germination rates were observed in the hypochlorite (98.0 ± 4.22%), control (97.0 ± 4.83%), water at room temperature (96.0 ± 6.99%) and water at 70º C (83.0 ± 29.08%) treatments. Thus, *Astronium fraxinifolium* diaspores do not present dormancy. During storage, the diaspores remained viable throughout the study period with high germination rates, except for the treatment in paper bags placed in the cold chamber, in which the diaspores lost their viability in the eighth month of storage. Therefore, this is not a recommended storage method for this species.
Keywords: plant propagation; tegument dormancy; vigor; viability; storage.

RESUMO

Este trabalho teve como objetivo avaliar a germinação e a capacidade de armazenamento de diásporos de *Astronium fraxinifolium*. Para o experimento de germinação, utilizaram-se seis tratamentos pré-germinativos: tratamento controle (diásporos intactos); diásporos imersos em água a temperatura ambiente (25º C) por 5 min.; diásporos imersos em água a 70° C por 5 min.; diásporos imersos em água a 100° C por 5 min.; diásporos imersos em solução de hipoclorito de sódio (1:1000) por 2 min.; e diásporos escarificados mecanicamente, com lixa nº 80. Para determinar a capacidade de armazenamento, foram testados dois diferentes tipos de embalagem (saco de papel permeável e vidro transparente) e duas condições ambientais (câmara fria e condições de laboratório). Foram realizados testes de germinação

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antes do armazenamento (tempo zero) e após 60, 120, 180, 240, 300 e 360 dias de armazenamento. Os efeitos dos diferentes tratamentos pré-germinativos e das condições de armazenamento na germinação dos diásporos foram avaliados por meio da ANOVA, seguida do teste de Tukey. Em relação aos tratamentos pré-germinativos, foram observadas altas taxas de germinação nos tratamentos com imersão em hipoclorito (98,0 ± 4,22%), controle (97,0 ± 4,83%), imersão em água destilada a temperatura ambiente (96,0 ± 6,99%) e imersão em água aquecida a 70ºC (83,0 ± 29,08%). Dessa forma, os diásporos de *Astronium fraxinifolium* não apresentaram dormência. No armazenamento, os diásporos permaneceram viáveis durante todo o período de estudo, apresentando altas porcentagens de germinação, com exceção do tratamento em saco de papel acondicionado em câmara fria, que perdeu a viabilidade no oitavo mês de armazenamento, não sendo, portanto, um método de armazenamento recomendado para esta espécie.
Palavras-chave: propagação vegetal; dormência tegumentar; vigor; viabilidade; armazenamento.

INTRODUCTION

The interest in the propagation of native species has intensified in recent years, highlighting the need of recuperating degraded areas and restoring the landscape (ALVES et al., 2004). The success of any restoration activity depends on many factors, such as knowledge of phytosociological aspects, population structure and species auto-ecology (VAN NOORDWIJK et al., 2007). This last factor involves the understanding of proper selection of seed trees (e.g. no symptoms of disease) and healthy seeds and fruits for seedling production, among others (FONSECA et al., 2001). Thus, the assessment of factors related to quality and quantity of seeds for planting is one of the most important aspects of restoration (VAN NOORDWIJK et al., 2007). Seed quality, in the absence of inputs and fertilizers, will enhance growth and productivity, particularly in degraded areas (SIMONS et al., 1994), and the appropriate amount of seed will ensure that the planting goals are achieved (VAN NOORDWIJK et al., 2007), both of which are essential aspects for projects of this nature.

The seed destined to sowing must be conserved to ensure the maintenance of physiological quality (GUEDES et al., 2012). Thus, when subjected to certain external (environment) and internal (intrinsic to the seeds) conditions, a viable seed initiates the germination process (BASKIN and BASKIN, 1998). However, seeds of several tropical species have some type of dormancy that prevents prompt germination, even in favorable environmental conditions (SOUZA and VÁLIO, 2001). This dormancy may originate from the integument, which may be physically limiting, acting as a barrier to the passage of gases and liquids (BILIA et al., 1998). Thus, to overcome this type of dormancy, mechanisms of tegument scarification that increase germination rate and can also speed up the germination process have been developed (NUNES et al., 2006), resulting in greater uniformity and survival (NASCIMENTO and OLIVEIRA, 1999).

Some species can maintain the viability of their seeds over a long time, whereas seeds of other species deteriorate rapidly, even when they are subjected to identical storage conditions (VILLELA and PERES, 2004). Deterioration is any degenerative change after the seed has reached its maximum quality (SANTOS et al., 2003) and is an irreversible and unavoidable process (AZEVEDO et al., 2003; CORTE et al., 2010). However, the speed of this process can be controlled (VILLELA and PERES, 2004). Therefore, storage controls the physiological quality of seeds and, consequently, preserves viability and maintains vigor for a longer period of time (AZEVEDO et al., 2003). The control of storage environment and use of adequate packaging, as well as the water level of seeds, can increase the longevity of stored seeds (VILLELA and PERES, 2004). Thus, ideal storage conditions are those that reduce the respiration and perspiration processes, thus decreasing embryo deterioration (PHARTYAL et al., 2002).

Brazil has a large number of plant species, but there is little information about the production of their seeds (AGUIAR et al., 2001). *Astronium fraxinifolium* Schott (Anacardiaceae) is a species considered a pioneer and selective xerophytic (LORENZI, 1992), so widely used in studies of recuperation of degraded areas. Popularly known as gonçalo-alves, it is a deciduous tree species (LORENZI, 1992; LEITE, 2002). It has broad occurrence in the savannas of central Brazil (Minas Gerais, Goiás and Mato Grosso states)

and in the Amazon forest (Pará state) (LORENZI, 1992; LEITE, 2002), and is also found in areas of Bolivia, Peru, Paraguay, Colombia, Venezuela and Guyana (LEITE, 2002; OLIVEIRA-FILHO, 2006). This species is of great economic importance, mainly due to its excellent quality of wood, which is extensively used in construction and shipbuilding (AGUIAR et al., 2001). Fruits with no epi- and mesocarp, from now on referred to as diaspores, are the dispersion units of this species (MORAES and PAOLI, 1996), and are produced in large quantities and characterized as tolerant to desiccation (LIMA et al., 2008).Although the conservation status of the species is considered "common" (OLIVEIRA-FILHO, 2006), it appears in the FAO list, as elaborated in the "Panel of Experts on Forest Gene Resources", as a priority for genetic conservation (ROCHE, 1987). However, deforestation, development of pests and diseases that attack the species and indiscriminate harvesting are the main causes of loss of genetic variability (AGUIAR et al., 2001).

Thus, as there is interest in the utilization of *Astronium fraxinifolium* for area restoration, the development of technologies for propagation and conservation of its diaspores is necessary. Studies on the germination of this species have been performed (MELO et al., 1979; NETTO and FAIAD, 1995; AGUIAR et al., 2001; LIMA et al., 2008). However, studies involving conditions and period of maintenance of the viability of *Astronium fraxinifolium* diaspores are scarce (see LIMA et al., 2008). Therefore, this study aimed to determine the behavior of these diaspores in relation to germination and conservation of physiological quality based on the following questions: (i) does germination potential of *Astronium fraxinifolium* diaspores vary in relation to pre-germination treatments applied and (ii) do time and storage conditions affect the germination of this species?

MATERIAL AND METHODS

Collection site and season

Ten mother trees that were at least 50 m apart were randomly selected for the collection, depending on the availability of diaspores, from Ouro Preto farm - AGROPOP, located in the Environmental Protection Area of Pandeiros river (EPA Pandeiros river), in Januária municipality, north of Minas Gerais state (15º 28' 47,6" S and 44° 22' 11,7" W), in September 2006. The area is a transition between Cerrado (savanna vegetation) and Caatinga (scrub dry forest) biomes with vegetation typical of savanna (Cerrado *sensu stricto*), with occurrence of riparian forest, dry forest, palm swamps, wetland, etc. (NUNES et al., 2009). Moreover, the collection site was in a deciduous dry forest. The climate is Aw (Köppen), with the annual average temperature and precipitation vary from 22 to 24° C/year and 900 to 1200 mm, respectively (NUNES et al., 2009).

Diaspores were collected using a cut-branch trimmer and a shading mesh (2 mm hole). After collection, the diaspores were packed in paper bags, properly identified and then sent to Laboratório de Ecologia e Propagação Vegetal at Universidade Estadual de Montes Claros- UNIMONTES, in Montes Claros, Minas Gerais state, where the germination and storage experiments were conducted. Within one week, the diaspores were screened in the laboratory and 60 diaspores were chosen per mother tree. During the screening, both predated (presence of insects or even trace of predation) and aborted (small size or apparent deformity) diaspores were discarded.

Germination and storage experiments

The diaspores were initially submitted to six treatments, with ten replications of ten diaspores per treatment, resulting in 100 diaspores for each treatment. The population sample (the lot of 100 diaspores) consisted of 10 diaspores per mother tree. This ensures randomness in the samples, with an equal number of diaspores from each plant used for the experiment (VILLELA e PERES, 2004). The pre-germination treatments used were: control treatment (C), where the diaspores remained intacts; diaspores immersed in distilled water at room temperature (25º C) for five minutes (AA); diaspores immersed in distilled water at 70° C for 5 minutes (A70); diaspores immersed in distilled water at 100° C for 5 minutes (A100); diaspores immersed in a solution of sodium hypochlorite and distilled water (1:1000) for 2 minutes (H); and mechanical scarification (L), using sandpaper (n° 80) until the endosperm, opposite to the hilum, was exposed.

For the storage experiment, diaspores were stored in different types of packaging and environmental conditions. Two types of packaging

were used: permeable paper bags and transparent glass pot. Two environments conditions were used for storage: a cold chamber (temperature of -4 ± 1° C and relative air humidity of 84 ± 2%) and *room conditions* (average temperature ranging between 18.6 and 30.7° C, and relative air humidity ranging between 66% and 85%). Thus, definitive treatments were room temperature in paper bag (PE), room temperature in glass jar (GE), cold chamber in paper bag (FP) and cold chamber in glass jar (FG). Each treatment consisted of 10 replications with 20 diaspores each. The tests were performed for zero, 60, 120, 180, 240, 300 and 360 days of storage, with time zero corresponding to the control treatment of the germination experiment.

Germination tests were conducted in a germination chamber (FANEM; model 347 CDG) with alternating temperature and light (30° C light/12 hours; 20° C dark/12 hours) in Petri dishes with paper-filter substrate moistened with distilled water (NUNES et al., 2006; 2008). Germinated diaspores were counted daily for 30 days. Diaspores were considered germinated when the radicle had protruded from the seed (LABOURIAU, 1983).

The effects of different pre-germination treatments on germination of *Astronium fraxinifolium* diaspores were evaluated by analysis of variance (ANOVA), and Tukey post-test (ZAR, 1996). ANOVA was also used to verify if the germination of diaspores varied among time and storage conditions. Therefore, the linear regression analysis (ZAR, 1996) was made between storage time and germination rates to verify the diaspores behavior under the storage period. For this, germination rates, from both tests, were arcsine-root percentagetransformed to homogenize the variances (SANTANA and RANAL, 2004). Treatment A100 (100º C/ 5 min) was excluded from the statistical analysis because no diaspores germinated after this treatment.

RESULTS AND DISCUSSION

Germination

Germination of *Astronium fraxinifolium* diaspores varied among different scarification treatments ($gl = 4$; $F = 31.277$; $p < 0.001$; $n = 50$). High germination was observed in the following treatments: immersion in hypochlorite (H), with mean of 98.0% (± 4.2%); control (C), with mean of 97.0% (± 4.8%); immersion in distilled water (AA), with mean of 96.0% (± 7.0%); and immersion in water heated to 70º C (A70), with mean of 83.0% (± 29.1%) (Table 1). On the other hand, diaspores that underwent mechanical scarification (L) showed low germination potential that differed significantly from other methods.

The germination peak for all tested treatments was observed between the 2nd and 3rd days after sowing (Table 1). Nevertheless, *Astronium fraxinifolium* diaspores germinated from the 2nd to 16th day after sowing and different germination behavior was observed among treatments. Thus, diaspores from treatment with water heated to 70º C (A70) germinated from the 2nd to 4th day, and diaspores from treatments distilled water (AA) and hypochlorite immersion (H) germinated from the 2nd to 5th day. However, diaspores mechanically scarified (L) and those from control treatment (C) germinated from the 2nd to 12th and 16th day after sowing, respectively. Therefore, germination rate and velocity found in treatments AA and H were high, but with very similar rates to treatment C (Table 1).

Imbibition is the process that characterizes the beginning of seed germination (COSTA et al., 2002), and species, number of pores distributed on the surface of the integument, water availability, temperature, hydrostatic pressure, water/seed contact area, intermolecular forces, chemical composition and physiological quality of seeds determine the speed of this mechanism of water absorption (POPINIGIS, 1977). In the absence of aryl containing chemical inhibitors to germination, the impermeable integument may be the main cause of lack of access to moisture (RAMOS and ZANON, 1986). Nevertheless, although integument destabilization can accelerate the germination process, all pre-germination treatments used in this study for *Astronium fraxinifolium* diaspores, except mechanical scarification, showed results similar to those in which diaspores were kept intact. Therefore, diaspores of the studied species do not show dormancy and consequently do not need treatment to increase their germination, given that intact, untreated diaspores showed germination potential statistically similar to treated diaspores. According to Carmello-Guerreiro and Paoli (2000), in *Astronium graveolens* Jacq., the mature diaspore coat is a very thin tissue and is considered weak and easily destabilized. Thus, the embryo can rupture the integument with no difficulty, even without scarification (CARMELLO-GUERREIRO and

TABLE 1: Mean germination percentage (± standard deviation), germination peak (day, mean percentage and standard deviation) and germination duration (days) of *Astronium fraxinifolium* Schott diaspores subjected to different pre-germination treatments, for 30 days in a germination chamber.

TABELA 1: Porcentagem media de germinação (± desvio padrão), pico de germinação (dia, porcentagem média e desvio padrão) e duração da germinação (dias) de diásporos de *Astronium fraxinifolium* Schott submetidos a diferentes tratamentos pré-germinativos, por 30 dias em câmara de germinação.

Treatments	Mean germination rate (X ± SD%)	Germination peak (day)	Germination peak (X ± SD%)	Germination duration (days)
Sodium hypochlorite (H)	98.0 ± 4.2	2^{sd}	72.0 ± 32.9	2^{sd} - 5th
Control (C)	97.0 ±4.8	3rd	34.0 ±20.7	2^{sd} - 16th
Water at room temperature (AA)	96.0 ±7.0	2^{sd}	8.0 ± 20.0	2^{sd} - 5th
Water at 70^{o} C (A70)	83.0 ± 29.1	2^{sd}	4.0 ± 25.4	2^{sd} - 4th
Mechanical scarification (L)	25.0 ± 13.5	3rd	9.0 ± 7.3	2^{sd} - 12th

PAOLI, 2000).

Treatments with immersion in hypochlorite, in distilled water at room temperature and in hot water resulted in high germination velocity for *Astronium fraxinifolium* diaspores, but did not differ from control treatment (Tabela 1). Given that one of the determining factors of germination is water absorption, the speed of this process depends on the permeability of the integument, temperature and seed chemical composition (PEREZ, 2004). Thus, the treatments were efficient in increasing the permeability of the integument to water. In contrast, the control treatment had low germination velocity and needed longer incubation.

In general, one we observe that among the methods for breaking dormancy, mechanical scarification was the only treatment that differed from the others, showing low germination rate. A species can respond differently to the treatments they have been subjected to, presenting different temporal distribution patterns of germination (BORGHETTI and FERREIRA, 2004). Therefore, the use of sandpaper as mechanical scarification was not effective for diaspores of the studied species, causing a reduction in their germination rate. This result may have occurred due to time of exposure and to scarification intensity, probably damaging the embryo and, consequently, the germination process (POPINIGIS, 1977). These results differ from those found by Nunes et al. (2008) for diaspores of aroeira (*Myracrodruon urundeuva* Allemão – Anacardiaceae), a species from the same botanical family and sympatric to *Astronium fraxinifolium*, in which the treatment "sandpaper" showed mean rates and germination velocity similar to the control treatment.

The immersion of dry seeds in boiling water can lead to wall rupture of the integument, which allows water to penetrate in the seed tissues and causes physiological changes and subsequent germination of the embryo (ALIERO, 2004). However, water at 100º C may have caused embryo death due to high temperature and the diaspore thin integument, explaining the lack of germination in this treatment. A similar result was found by Alves et al. (2004) in *Bauhinia divaricata* L. (Fabaceae) seeds, in which low rates of emergence were obtained with seeds immersed in hot water at 80° C. In addition, Aliero (2004) observed that exposure of *Parkia biglobosa* Jacq. Benth (Fabaceae) seeds to hot water for a period longer than four seconds significantly reduces germination rate. Aliero (2004) also suggests that contact with boiling water for a long period can destroy the embryo.

Storage

There were significant differences in the germination of *Astronium fraxinifolium* diaspores among conditions ($gl = 3$; $F = 50.997$; $p < 0,001$; $n = 240$) and months of storage ($gl = 5$; $F = 37.120$; $p < 0,001$; $n = 240$), and in the relationship between conditions and time ($gl = 15$; $F = 28.554$; $p < 0,001$; $n = 240$) (Figure 1). Glass container in a cold chamber (FG) treatment

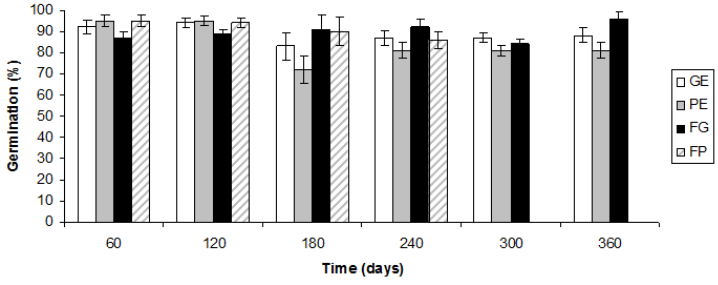

FIGURE 1: Mean germination percentage (± standard error) of *Astronium fraxinifolium* Schott diaspores subjected to different conditions and time of storage. GE = glass pot in room temperature; PE = paper bag in room temperature; FG = glass pot in cold chamber; and FP = paper in cold chamber.

FIGURA 1: Porcentagem media de germinação (± erro padrão) de diásporos de *Astronium fraxinifolium* Schott submetidos a diferentes condições e tempo de estocagem. GE = pote de vidro em temperatura ambiente; PE = saco de papel em temperatura ambiente; FG = pote de vidro em câmara fria; e FP = saco de papel em câmara fria.

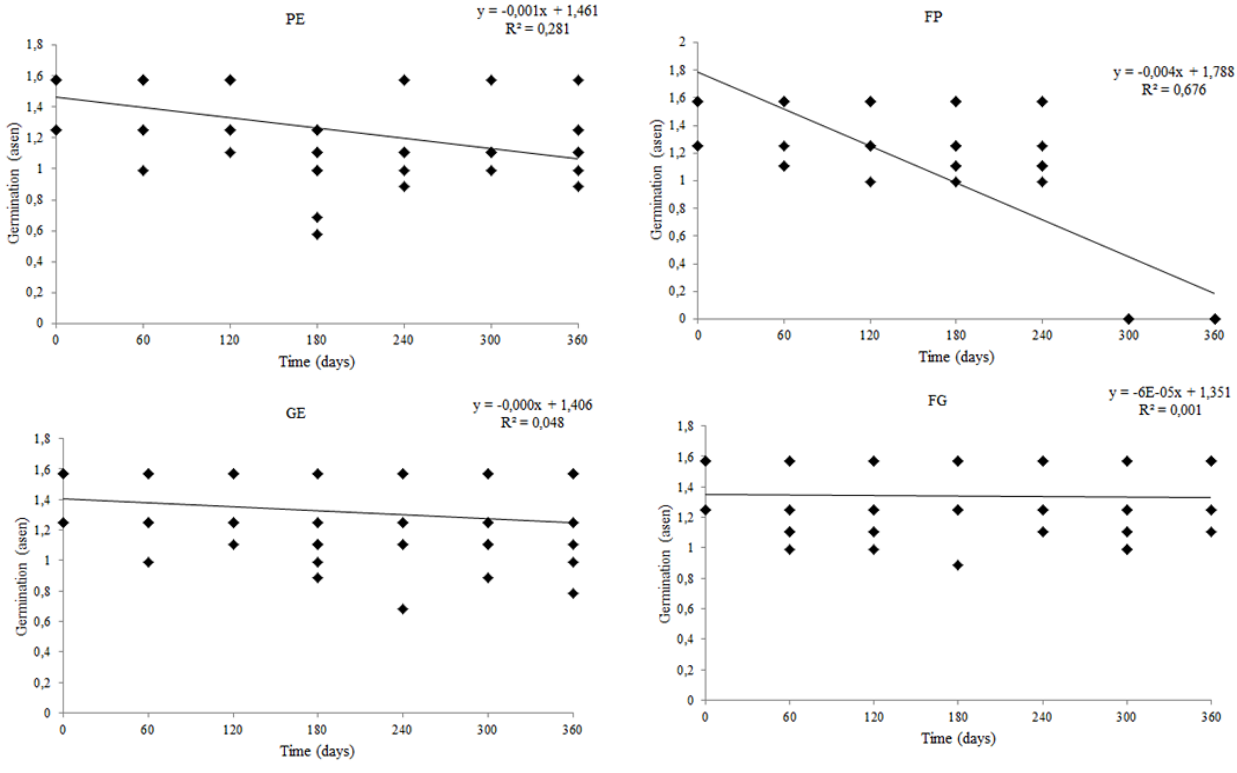

FIGURE 2: Relation between storage period (days) and germination rate (arcsine-root percentage) of *Astronium fraxinifolium* Schott diaspores subject to different store conditions and time. PE = paper bag in room temperature; FP = paper in cold chamber; GE = glass pot in room temperature; and FG = glass pot in cold chamber.

FIGURA 2: Relação entre o período de armazenamento (dias) e a taxa de germinação (arcosseno da raiz da porcentagem) dos diásporos de *Astronium fraxinifolium* Schott submetidos a diferentes condições e tempo de armazenamento. PE = saco de papel em temperatura ambiente; FP = saco de papel em câmara fria; GE = pote de vidro em temperatura ambiente; e FG = pote de vidro em câmara fria.

showed fluctuations in germination rates during storage period, with the highest germination rate recorded in the last test (96.0 ± 6.99%) at 360 days of storage. On the other hand, paper container in cold chamber (FP) treatment resulted in no germinated diaspores in the last two tests times, at 300 and 360 days. Moreover, the other two treatments, room conditions in glass and paper containers (glass pot in room temperature - GE and paper bag in room temperature - PE), also showed small fluctuations during storage, and paper bag in room temperature (PE) treatment maintained a constant germination rate in the last three tests at 240, 300 and 360 days.

According to Lorenzi (1992), *Astronium fraxinifolium* diaspores can be stored and have their viability maintained for a period of up to four months. In contrast, we observed that diaspores from this species can be viable for at least one year of storage because there was germination throughout the storage period (12 months). In a study performed by Lima et al. (2008), storage in a paper bag at room temperature allowed *Astronium fraxinifolium* diaspores to remain viable for 15 months, with germination rates close to the original one throughout the study. Thus, despite variations of temperature and quite high relative humidity in room conditions, which varies throughout the day and according to the month (KANO et al., 1978), diaspores did not change their behavior in response to different types of packaging when stored at room temperature.

There was a tendency to decrease the germination rate for the diaspores packed in paper bags after 240 days of storage, for those placed in cold chamber ($p < 0.001$; $R^2 = 0.68$) and at room temperature ($p < 0.01$; $R^2 = 0.28$). The higher porosity of the paper bag may be responsible for the loss of viability of *Astronium fraxinifolium* seeds in these conditions (KANO et al., 1978).

In the cold chamber, diaspores showed differences in germination rate when different packaging types (glass jar and paper bag) were compared. Diaspores stored in glass containers had higher germination rates compared to those in paper packaging, in which there was no germinated diaspore in the last two periods of storage tested (300 and 360 days). This behavior was similar for seeds of *Senegalia polyphylla* (DC.) Britton e Rose (Fabaceae), in which there physiological quality was maintained for at least two years when stored in a cold chamber and packed in glass containers (ARAÚJO-NETO et al., 2005). In studies with seeds of *Ocotea porosa* (Mez) L. Barroso (Lauraceae), Tonin and Perez (2006) reported that glass containers are not recommended for this species. This result was different from ours, in which treatment FG (cold chamber and glass container) had the highest germination rate. Piña-Rodrigues and Jesus (1992) observed that *Cedrela angustifolia* S. Et. Moc. (Meliaceae) seeds maintained higher viability when stored in dry-cold-chamber than in room conditions.

CONCLUSION

For germination tests, *Astronium fraxinifolium* diaspores do not need pre-germination treatments given that this species has no dormancy. However, treatments with immersion in hypochlorite, in distilled water, at room temperature and in hot water increased the speed of the germination process, however did not differ from control. The storage of *Astronium fraxinifolium* diaspores in both glass and paper at room conditions and in glass at freezer conditions is recommended. In these conditions, the diaspores present high germination percentages during one year of storage. However, the diaspores completely lost their viability in paper bags at freezer conditions after 240 days of storage.

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