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Influence of the functional traits of seeds on germination dynamics and morphofunctional pattern of the seedlings

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ABSTRACT. Aiming to compare morphofunctional features related to dispersion and establishment of plants, this study evaluated seed biometry and biomass, as well as germination and emergence potential in eight plant species native to the Semideciduous Seasonal Forest. Biometric measurements and biomass of seeds were obtained with a caliper and a precision scale. Afterwards, they were subjected to a germination test in BOD chambers under constant temperature and 12 hours photoperiod, and sown in styrofoam trays containing organic substrate and sand, kept in full sunlight. Data from germination and emergence was subjected to cluster analysis, according to the functional morphotype of the seedling. Most species presented seeds with a rounded shape, uniform staining at the mature stage, varied coat consistency, two types of dispersal syndromes (anemochory and zoochory) and four morphofunctional patterns in developing seedlings (crypto-hypogeal-storers, crypto-epigeal-storers, phanero-epigeal-storers and phanero-epigeal-foliaceous). The species belonging to group I (*Albizia niopoides*, *Cedrela fissilis*, *Pterogyne nitens* and *Randia ferox*) have foliaceous photosynthetic cotyledons and group II (*Cabralea canjerana*, *Eugenia pyriformis*, *Inga laurina* and *Poecilanthe parviflora*) have a reserve cotyledon. Species of group I had higher means in the percentage and rate of germination, less variability and amplitude of days for seedlings emergence. On the other hand, species of group II showed higher mean values in seed biometry and biomass. Thus, seeds with foliaceous cotyledons have an optimized development because of luminosity, whereas seeds with reserve tend to have slower development, remaining at the seedling stage for a longer time.

Keywords: cotyledon; emergency; pioneer plants; reserve.

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Introduction

The Atlantic Forest is one of the Brazilian biomes that is home to a high tropical biodiversity, but because of anthropic intervention, it is one of the most endangered ecosystems (Ribeiro, Metzger, Martensen, Ponzoni, & Hirota, 2009). This is why the Atlantic Forest is a hotspot, being considered a priority for biodiversity conservation (Muylaert et al., 2018). Currently, it is estimated that most of this forest is reduced to fragmented remnants, which is remarkably observed for the phytophysiology of the Semideciduous Seasonal Forest (Troppmair, 1990), also known as the “Inland Atlantic Forest”. Thus, knowing and understanding the ecological dynamics of plant spread in this biome can contribute to the management and conservation of native species.

One of the largest remnants of the Semideciduous Seasonal Forest is the Conservation Unit (UC), “Estação Ecológica do Caiuá”(EEC), located in the municipality of Diamante do Norte, the northwestern region of the State of Paraná. It was created in 1994 to protect part of the regional ecosystem. EEC belongs to the Lower Paranapanema River Basin, with part of the area occupying the banks of the Rosana Hydropower Plant (Rosana HPP) Reservoir, with a great diversity of flora and fauna (Instituto Ambiental do Paraná [IAP], 2009).

The functional strategies used by plants, from the dispersal process, germination to seedling establishment, contribute to the maintenance of biological diversity and the natural regeneration of forests (Lopes, Osman, & Piedade, 2012). In this context, Cosmo, Nogueira, Lima, and Kuniyoshi (2010) consider

relevant the study of seed and seedling morphofunctionality for understanding plant adaptations to the environment and the continued survival of species.

Tropical trees exhibit high variability regarding seed morphofunctional characteristics, since the absence or presence of energy reserves determine cotyledon function, which consequently affect seedling establishment and directly influence the successional dynamics of the species (Andrade, Bruno, Oliveira, & Silva, 2010). In this sense, it is necessary to expand studies including silvicultural information on forest species, because according to Ribeiro et al. (2009), rational management based on scientific, social and economic interest depends on the knowledge of the regenerative and productive capacity of forests. Therefore, this study aimed to evaluate seed and seedling morphofunctional characteristics in eight tree species native from the Semideciduous Seasonal Forest, and to set a relationship of functional morphotypes and biometric parameters with potential seed germination and seedling emergence.

Material and methods

Collection area and identification of the plant material

Fruit and seed collection of the species selected for the experiments were conducted at Caiuá Ecological Station (52°49' to 52°53'W and 22°34' to 22°37' S), located in Diamante do Norte, State of Paraná, Brazil. This area has a Cfa-mesothermal climate without long periods of drought and rainfall, with low winter temperatures and hot summers (Maack, 2012), whose vegetation is in the Atlantic Forest domain and the phytophysognomy of the Semideciduous Seasonal Forest, on dystrophic sandy soils (IAP, 2009).

The analyzed botanical material was collected from at least five mother trees in preserved forest cover stretches, in the years 2015 and 2016, during the reproductive period, when the fruits were ripe. The species selection was randomized according to the availability of sufficient seeds for morphological and biometric assessment and germination bioassays. Moreover, the presence of a larger number of species of the family Fabaceae because it is the second largest, in number of species, in the Atlantica Forest domain (Stehmann, Forzza, Sobral, & Kamino, 2009) and the most representative in the study area. The species used in this study were compared to reference material deposited in the Herbarium of the *Universidade Estadual de Maringá* (HUEM), according to Table 1.

Table 1. List of species collected and analyzed.

Species	Family	HUEM
<i>Albizia niopoides</i> (Spruce ex Benth) Burkart	Fabaceae	16.415
<i>Cabralea canjerana</i> (Vell.) Mart.	Meliaceae	19.186
<i>Cedrela fissilis</i> Vell.	Meliaceae	16.487
<i>Eugenia pyriformis</i> Cambess.	Myrtaceae	22.508
<i>Inga laurina</i> (Sw.) Willd.	Fabaceae	22.795
<i>Poecilanthus parviflora</i> Benth.	Fabaceae	23.226
<i>Pterogyne nitens</i> Tul.	Fabaceae	22.748
<i>Randia ferox</i> (Cham. & Schltdl.) DC	Rubiaceae	21.413

Morphological, biometric and seed biomass assessments

The external seed morphology was analyzed with the aid of the Leica ICC50 stereoscopic microscope coupled to a digital camera. To support the classification and diagnosis of structures present in the seed samples, works by Souza, Moscheta, and Mourão (2006) and Gonçalves and Lorenzi (2007) were consulted.

For biometric evaluation, the length and diameter of 100 seed units of each species, randomly chosen, were analyzed with a digital caliper (accurate to 0.1 mm). Length was considered to be the region between the basal and apical portion, and diameter was the equatorial line of the seed. Also, fresh biomass was determined using the precision analytical balance (0.01 g), using 25 seeds with four repetitions. The calculation of the means and coefficients of variation was analyzed by the R software (R Team Core, 2016) and the statistical difference between the groups of plants obtained by Tukey's test ($\alpha = 5\%$).

Assessment of germination bioassays and seedling initial growth

Seeds were taken to the Experimental Laboratory of Plant Physiology of the *Universidade Estadual de Maringá* (UEM), and initially subjected to the disinfestation process, using 50% commercial sodium

hypochlorite solution and then placed in distilled water, with manual agitation, according to the indications of Brasil. Ministério da Agricultura, Pecuária e Abastecimento (2009).

For germination bioassays, four repetitions of 25 seeds were used, totaling 100 sampling units for each species evaluated. Seeds were placed in Petri dishes containing two filter paper discs moistened with distilled water and kept in a germination chamber (BOD), at a constant temperature of 25°C and 12 hours photoperiod. The determination to use 25°C for germination tests was based on studies by Brancalion, Novembre, and Rodrigues (2010), which indicate the use of a constant temperature of 25°C for the germination of seeds of Brazilian tree species from the Atlantic Forest. Germination was evaluated every 24 hours and the taproot protrusion (2 mm) was used as a criterion for germination. In the end it was obtained the germination percentage (GP), the germination speed index (GSI), according to Maguire (1962), and the mean germination time (MGT), according to Ferreira and Borghetti (2004).

To obtain the seedlings, 100 units of seeds of each species were sown in Styrofoam trays containing medium-grained sand and Provaso organic fertilizer, as substrate, at a ratio of 2: 1, respectively. Sowing was conducted in a greenhouse under light condition and with manual and periodic watering. After seedling emergence, the functional type of the cotyledon was visually verified, based on Souza (2009). Also, the average number of days for expansion of cotyledon leaves and the first pair of eophylls was calculated. The obtained data were subjected to cluster analysis, according to the functional morphotype of seeds at seedling emergence. Group I was composed of species with foliaceous cotyledons, while group II consisted of species with reserve cotyledons. The comparison of means between the two groups was analyzed by Tukey's test at 5% significance level.

Results

The modes of dispersal were associated with the morphological and functional structure of the diaspores, and the occurrence of longitudinal crack dehiscence in the dry and dehiscent legumes of *Albizia niopoides* and *Poecilanthe parviflora* (Figure 1A and B) (Table 2), the presence of membranous wings in the seeds of *Cedrela fissilis* (Figure 2G) and in samara of *Pterogyne nitens* (Figure 1C), fleshy and succulent drupe of *Eugenia pyriformis* (Figure 1D) (Table 2), sarcotesta in seeds of *Cabralea canjerana* (Figure 2E) and *Inga laurina* (Figure 1E) and arylid mass involving *Randia ferox* seeds (Figure 1F).

Out of the eight species evaluated, six exhibited round shaped seeds (Figure 2A, B, C, D, E, F), except for *Cedrela fissilis*, whose seeds are flat with tapered ends (Figure 2G) and *Randia ferox*, with triangular ends (Figure 2H), besides presenting uniform color on their surfaces at the mature stage. The seed coat consistency and fruit morphological characteristics were varied among species (Table 2).

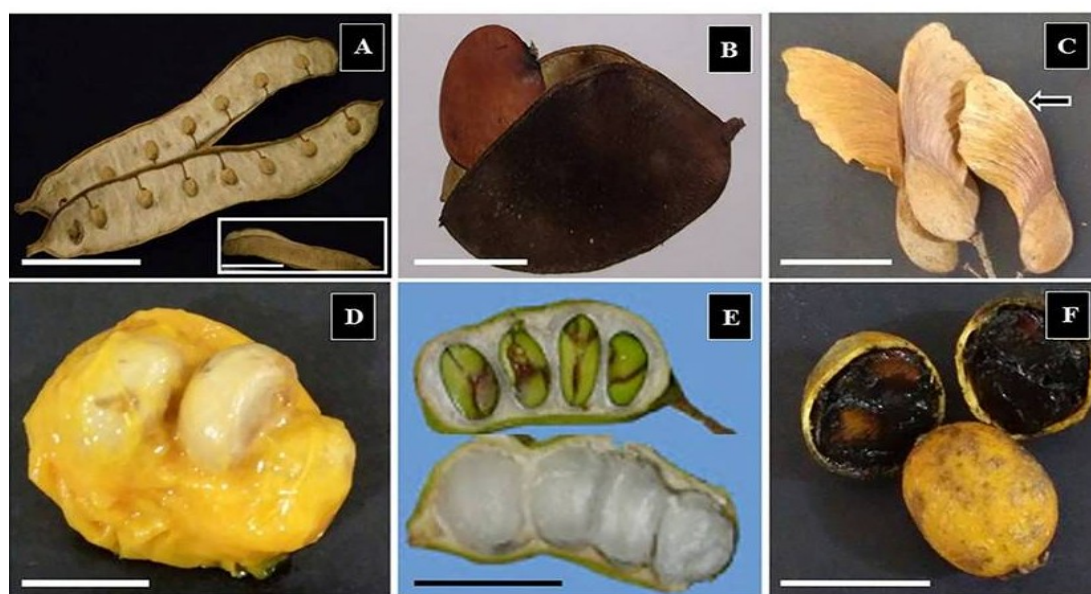


Figure 1. Fruit and seeds of (A) *Albizia niopoides*; (B) *Poecilanthe parviflora*; (C) *Pterogyne nitens*, winged fruit; (D) *Eugenia pyriformis*; (E) *Inga laurina*; (F) *Randia ferox*. Scale: 1 cm. The arrow indicates the wing of the samara.

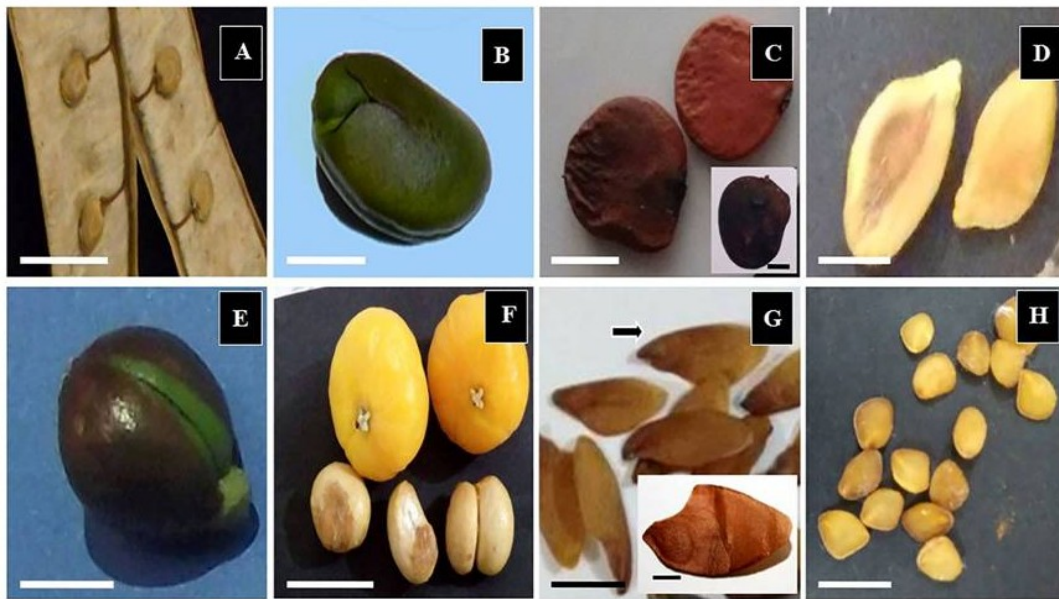


Figure 2. Seed morphology of the investigated species: (A) *Albizia niopoides*: oval and brownish; (B) *Inga laurina*: oblong and elongated, dark green; (C) *Poecilanthe parviflora*: circular and flat, red; (D) *Pterogyne nitens*: elliptical and brownish; (E) *Cabralea canjerana*: ovoid and greenish; (F) *Eugenia pyriformis*: rounded and thick, brownish; (G) *Cedrela fissilis*: flat and tapered at the ends; (H) *Randia ferox*: discoid triangulated, golden-yellow. Scale: 1 cm.

Table 2. Seed coat and fruit classification.

Species	Seed coat	Fruit
<i>Albizia niopoides</i>	Coriaceous	Legume, dry, dehiscent, polyspermic
<i>Cabralea canjerana</i>	Coriaceous	Capsule, fleshy, dehiscent, polyspermic
<i>Cedrela fissilis</i>	Membranaceous	Capsule, dry, dehiscent, polyspermic
<i>Eugenia pyriformis</i>	Coriaceous	Drupe, fleshy, indehiscent, polyspermic
<i>Inga laurina</i>	Coriaceous	Legume, fleshy, indehiscent, polyspermic
<i>Poecilanthe parviflora</i>	Coriaceous	Legume, dry, dehiscent, monospermic (sometimes bispermic)
<i>Pterogyne nitens</i>	Cartaceous	Samara, dry, indehiscent, monospermic
<i>Randia ferox</i>	Cartaceous	Berry, fleshy, indehiscent, polyspermic

Most of the evaluated species presented phanerocotyledonary seedlings and epigeal, except for *Eugenia pyriformis*, which is cryptocotyledonary and hypogeal. Four morphofunctional patterns were identified in developing seedlings, which were grouped into two groups (group I and group II) considering the cotyledon consistency. Thus, *Albizia niopoides*, *Cedrela fissilis*, *Pterogyne nitens* and *Randia ferox* (phanero-epigeal-foliaceous) (Figure 3A, B, C and D) composed group I, while *Eugenia pyriformis* (crypto-hypogeal-storer) (Figure 3E); *Poecilanthe parviflora* (crypto-epigeal-storer) (Figure 3F), *Inga laurina* and *Cabralea canjerana* (phanero-epigeal-storers) (Figure 3G and 3H) formed group II.

Comparative analyses between seed biometrics and biomass data (Table 3) allowed to infer that seeds with reserve (group II) had relatively higher mean biomass (10.18 g) and biometry (12.6 mm for length and 9.5 mm for diameter), when compared to the seeds with low reserve (group I), which obtained values averages around 1.30 g biomass, 8.5 mm in length and 5.4 mm in diameter.

The germination percentage in *Albizia niopoides*, *Cedrela fissilis*, *Inga laurina*, *Pterogyne nitens* and *Randia ferox* was significantly higher than the germination values shown in *Cabralea canjerana*, *Eugenia pyriformis* and *Poecilanthe parviflora*, which in turn can be considered low, reaching 56%, 20% and 46%, respectively. Moreover, there was a longer mean time to start the germination process in *A. niopoides*, *C. canjerana*, *E. pyriformis* and *P. parviflora*, when compared to the other evaluated species. In turn, the germination speed index was proportional to germinability (Table 4).

Regarding the mean values obtained from the variables analyzed in the seed germination process, there was a significant difference in the proportion and speed of germination between the groups of plants. The species of group I had a relatively higher value in germination percentage (GP) and germination speed index (GSI) compared to the mean value obtained by seeds of group II species. Considering the mean germination time (MGT), there was no significant difference between the groups of plants (Table 5).

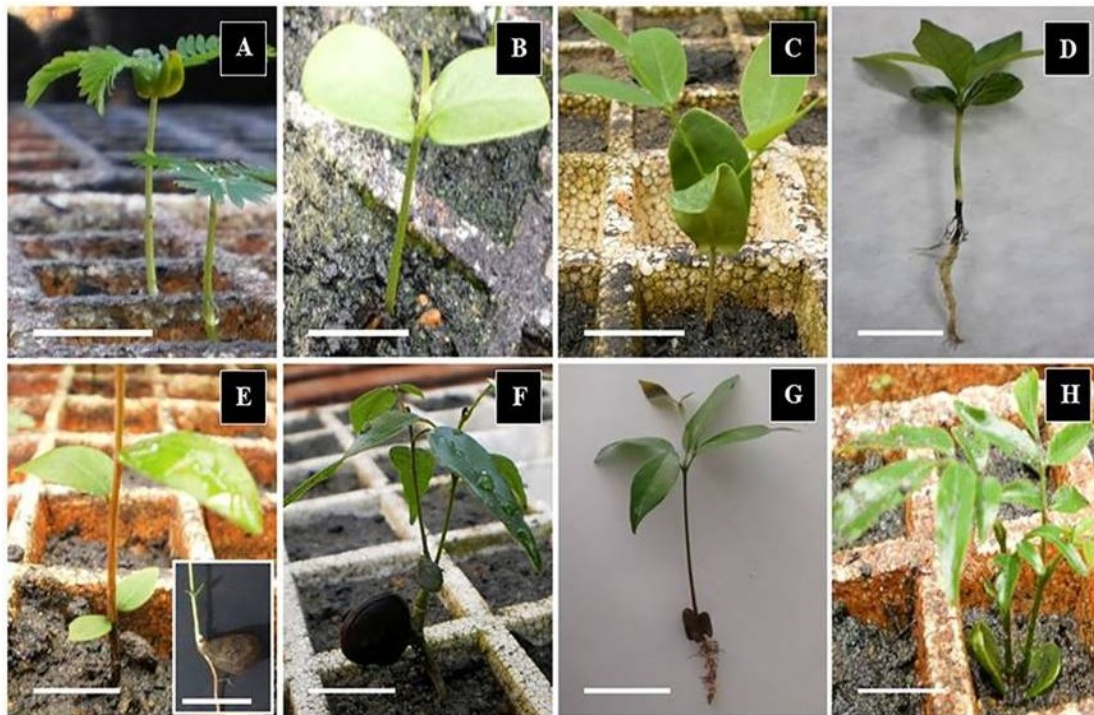


Figure 3. Seedling morphology. Phanero-epigeal-foliaceous species: *Albizia niopoides* (A); *Cedrela fissilis* (B); *Pterogyne nitens* (C); *Randia ferox* (D). Cryptohypogeal-storer species: *Eugenia pyriformis* (E). Cryptohypogeal-storer species: *Poecilanthe parviflora* (F). Phanero-epigeal-storer species: *Inga laurina* (G); *Cabralea canjerana* (H). Scale: 1 cm.

Table 3. Mean values of biometry and biomass of seeds of the evaluated species.

Species	Length (mm)				Diameter (mm)				Fresh biomass (g)			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
<i>Albizia niopoides</i>	4.5	7.3	6.32	0.44	2.8	4.9	3.86	0.41	0.87	0.96	0.93	0.04
<i>Cabralea canjerana</i>	5.8	11.5	7.82	0.98	5.4	7.9	6.55	0.5	5.43	5.66	5.58	0.1
<i>Cedrela fissilis</i>	7.3	14.3	9.5	1.2	4.4	7.3	5.79	0.61	0.98	1.12	1.06	0.1
<i>Eugenia pyriformis</i>	10.5	17	13.12	1.28	8.7	14.8	11.67	1.16	23.97	29.8	26.61	0.53
<i>Inga laurina</i>	12.2	17.7	15.34	1.13	4.4	9.7	7.14	0.97	1.85	1.98	1.91	0.06
<i>Poecilanthe parviflora</i>	12.4	17	14.43	0.97	9.8	15.3	12.82	1.04	5.99	7.08	6.62	0.47
<i>Pterogyne nitens</i>	8.6	12.4	10.96	0.76	5.2	7.2	6.17	0.43	1.57	1.73	1.63	0.07
<i>Randia ferox</i>	5.4	8.4	7.23	0.59	3.9	7.1	5.79	0.64	1.49	1.71	1.6	0.09

Min = Minimum value; Max = Maximum value; SD = Standard deviation.

Table 4. Mean values of germination data of the evaluated species.

Species	GP (%)				MGT (days)				GSI			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
<i>Albizia niopoides</i>	68	100	88	13.86	11.08	15.95	13.97	1.51	2.55	5.17	3.52	1.16
<i>Cabralea canjerana</i>	40	76	56	15.66	7.7	9.2	8.36	0.68	1.43	2.84	1.98	0.6
<i>Cedrela fissilis</i>	80	96	88	7.3	6.39	8.14	6.84	0.86	3.77	4.72	4.15	0.41
<i>Eugenia pyriformis</i>	4	32	20	11.77	19.5	22.6	21.11	1.43	0.04	0.39	0.24	0.15
<i>Inga laurina</i>	68	96	86	12.44	2.68	4.58	3.54	0.91	6.27	11.12	9.04	2.05
<i>Poecilanthe parviflora</i>	24	68	46	18.03	7.64	10.41	8.75	1.31	1.3	2.62	2.07	0.62
<i>Pterogyne nitens</i>	80	100	92	8.64	1.47	1.62	1.54	0.06	16.03	22.17	18.84	2.6
<i>Randia ferox</i>	76	96	85	8.7	3.13	4.73	3.96	0.81	2.52	6.51	4.87	1.82

PG = Porcentagem de germinação; TMG = Tempo médio de germinação; IVG = Índice de velocidade de germinação. Min = Valor mínimo; Max = Valor máximo; D.p = Desvio padrão. GP = germination percentage; MGT = mean germination time; GSI = Germination speed index. Min = Minimum value; Max = maximum value; SD = Standard deviation.

Table 5. Mean values of germination and emergence of the species of Group I and II.

Groups	GP (%)	MGT (days)	GSI	Cotyledon emergence (days)	Eophyll expansion (days)
Group I	88.25 a	6.50 a	7.85 a	7.95 b	13.47 b
Group II	52.00 b	10.44 a	3.32 b	13.8 a	26.32 a

*Different letters in the same column indicate significant difference by Tukey' test at 5%. GP = germination percentage; MGT = mean germination time; GSI = Germination speed index.

In the process of emergence of cotyledons and eophylls, group I species presented lower mean days for their expansion, when compared to the values obtained by the group II species (Table 5), i.e., foliaceous cotyledon emerge earlier and their expansion is more homogeneous. It was evidenced that *Albizia niopoides*, *Cedrela fissilis*, *Pterogyne nitens* and *Randia ferox* (group I) required 12 days for the emergence of cotyledons and 18 days for the complete expansion of eophylls, while *Cabralea canjerana*, *Eugenia pyriformis*, *Inga laurina* and *Poecilanthe parviflora* (group II) took 20 days for cotyledon emergence and 35 days for eophyll expansion.

Discussion

In typically anemochoric diaspores, the longitudinal dehiscence in dry fruit associated with membranous wings, as in seeds of *Cedrela fissilis* and fruit of *Pterogyne nitens*, facilitates the action of wind as a dispersing element, as reported by Oliveira and Barbosa (2014). Bernasol and Ribeiro (2010) stated that plants with anemochoric syndrome disperse their seeds over large distances from the mother plant, representing an advantage for their establishment. Still, they associate anemochory with the pattern of random spatial distribution, since adult plants spread their seeds at random. On the other hand, in zoochoric seeds, the presence of nutritive and/or attractive substances for animals was observed, such as succulent pericarp, edible sarcotesta and aril. *Cabralea canjerana*, *Eugenia pyriformis*, *Inga laurina*, and *Randia ferox* seeds present characteristics that favor zoochory, which was also reported by Leyser, Viniski, Donida, Zanin, and Budke (2009) and Leão, Lima, Pinto, and Paiva (2012). These structures are consumed by several avifauna and mammal species, which can ingest and regurgitate seeds, culminating in an endozoochoric process (Deminicis et al., 2009). The spatial distribution of zoochoric plants is estimated to be aggregate, since most of the time seeds are deposited in specific locations, i.e., near the animal feeding microsites (Bernasol & Ribeiro, 2010). Considering the species with zoochoric dispersal, the spherical shape and rigid consistency of the seminal coats, verified in *C. canjerana*, *E. pyriformis* and *R. ferox*, favor the chances of plant survival, as they are less susceptible to chewing damage and often pass faster through the digestive tract of animals (Deminicis et al., 2009).

Seeds with rigid coats, as occurs in *R. ferox* and *P. nitens*, prevent the entry of water and gases, which hinders germination, while the coriaceous coat found in *A. niopoides*, *C. canjerana*, *E. pyriformis*, *I. laurina*, *P. parviflora* and the membranous coat in *C. fissilis* are more sensitive to water diffusion, resulting in a more incipient embryonic imbibition. Therefore, Alves, Sader, Bruno, and Alves (2005) highlighted the importance of the use of color and consistency of seminal integuments, as criteria for recognizing physiological maturity point and seed imbibition behavior, since they are factors that influence the germination process. Thus, coloration can be used to manage the seeds in the period they are most viable and to overcome tegument dormancy through mechanical scarification or pre-imbibition treatments, as suggested by Oliveira et al. (2008).

Seed size and biomass influence the number produced by the species, since smaller seeds are produced in larger quantities and tend to become dormant, forming seed banks in the soil, while larger seeds can germinate in shaded areas and having more reserves, they tend to form a seedling bank (Wiesbauer, Giehl, & Jarenkow, 2008). Also, smaller seeds usually correspond to early successional species, which invest in the largest possible proportion of seminal units, to the detriment of lower biomass. On the other hand, species that produce seeds rich in nutritional reserves are predominantly secondary, with high energy content, which gives them greater biomass (Dresch, Scalón, Masetto, & Vieira, 2013). Thus, seeds of higher biomass, have well-formed embryos and, therefore, give rise to more vigorous seedlings that survive longer under environmental conditions that do not yet allow the use of soil nutritional components and also the photosynthetic process (Carvalho & Nakagawa, 2012).

The germination percentage and germination speed index are influenced by exposure to radiation. Due to this, the species without reserves, whose cotyledons are foliaceous, perform the photosynthetic process earlier, culminating in rapid germination and growth, representing a strategic advantage in open, degraded or forest clearing environments (Ressel, Guilherme, Schiavini, & Oliveira, 2004). In contrast, reserve cotyledons, which is the condition present in group II species, provide energy and nutrients for seedling development, while photosynthate production is limited (Soriano et al., 2011). As a result, *C. canjerana*, *E. pyriformis*, *I. laurina* and *P. parviflora* had slower initial development of their seedlings, from the germination process to the complete expansion of eophylls. Garwood (1996) considers that these

characteristics are common in shade-tolerant species, since the ideal conditions for germination and seedling establishment occur in understory environments, where the incidence of light is limited.

Light requirement for germination is interpreted as a way for the seed to recognize a canopy opening favorable to subsequent seedling growth (Dutra; Medeiros-Filho, & Diniz, 2008), as seeds with low reserve, a condition observed in group I, cannot maintain long-term seedling development under shade conditions. On the other hand, seeds of group II species, which exhibited considerably higher biomass in their seeds (Table 3), due to the presence of energy reserves, other abiotic factors, such as temperature alternation and soil moisture content, have a stronger influence to signal the germination process than the exposure to light (Matos & Landim, 2016).

The morphological differences exhibited in the seminal structure are related to ecological functionality adapted to variations in environmental conditions (Andrade et al., 2010). Thus, the morphological suitability of seedlings to environmental conditions may interfere with the reproductive success of the species (Lobo, Santana, Salomão, Rehbein, & Wielewicki, 2014). Therefore, the demand for technical data on native species biology, especially on optimal germination and optimal development conditions, is of utmost importance for the success of degraded area recovery program, requiring further studies to understand the structure of forest dynamics.

Conclusion

The species that produce large seeds with higher biomass content have slow initial development and are dependent on seminal reserves, as found in *Cabralea canjerana*, *Eugenia pyriformis*, *Inga laurina* and *Poecilanthe parviflora*, which form a seedling bank in the soil and are suitable to compose the forest understory, being compatible with the behavior of non-pioneer plants. In contrast, smaller seeds are produced in large quantities and have low biomass. These characteristics were found in *Albizia niopoides*, *Cedrela fissilis*, *Pterogyne nitens* and *Randia ferox*, whose germination and development were optimized as a function of light. The early production of photoassimilates was observed at the seedling stage. Such species can compose clearings, forest edges and open environments, where there is a high incidence of light, being compatible with the ecological behavior of pioneer plants.

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