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**Atributos das sementes e germinação de espécies arbóreas:
perspectivas para restauração ecológica**

Porto Alegre

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Dissertação apresentada ao Programa de Pós-Graduação em Botânica da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para a obtenção do título de Mestres em Botânica.

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**“Mulheres, tentaram nos enterrar
mas não sabiam que éramos sementes.”**

(Adaptado do Provérbio Mexicano)

Agradecimentos

Agradecer só a algumas pessoas talvez possa indicar um esquecimento da ecologia. Esquecer da ciclicidade dos processos. Esquecer que tudo esta interligado. Esquecer que um processo para ocorrer não depende apenas de um fator. Aliás, temos cada vez mais usado análises multivariadas para entender melhor o que mais afeta um processo, e sabemos o quanto é difícil, pois afinal tudo ocorre por um conjunto de fatores. Talvez para conseguir selecionar alguns nomes e agradecer nessa dissertação, eu deveria escrever outro artigo, e logo, precisaria de mais tempo.

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Resumo

A degradação florestal tem aumentado significativamente nas últimas décadas e gerado impactos ambientais consideráveis, exigindo o estabelecimento de técnicas economicamente viáveis e eficazes de restauração florestal. Atualmente tem sido proposta a semeadura direta como uma alternativa ao plantio de mudas devido ao seu baixo custo. No entanto, ainda são poucos os trabalhos com semeadura direta que objetivem selecionar espécies que apresentam maiores porcentagens de emergência e sobrevivência. Diante disso, nós realizamos um estudo de semeadura direta com espécies arbóreas nativas da Floresta Estacional Semidecidual, no sul do Brasil, com o objetivo de: (1) determinar se atributos reprodutivos (massa, forma e conteúdo de água da semente) influenciam na germinação; (2) testar a efetividade da semeadura direta em tratamentos com e sem cobertura de palhada, sementes enterradas e não enterradas e (3) estabelecer critérios para a seleção de espécies para restauração ecológica. Foram coletadas sementes de 17 espécies, testadas a germinação em laboratório e, destas, 12 foram semeadas em campo. Foram medidos a forma, massa e conteúdo de água das sementes. Estes atributos foram relacionados com a taxa de germinação, tempo médio de germinação e índice de velocidade de germinação para as espécies testadas em laboratório. Na semeadura direta foram calculados a emergência, estabelecimento e sobrevivência e comparado as diferenças entre três tratamentos: enterrada com cobertura, enterrada sem cobertura e não enterrada com cobertura. Em laboratório o formato das sementes teve efeito sobre o índice de velocidade germinação, indicando que as sementes achatadas germinam mais rapidamente.

Na sementeira direta, as sementes enterradas e com cobertura de palhada apresentaram maiores taxas de germinação (52% para *Allophylus edulis*), porém não houve efeito significativo para o estabelecimento e a sobrevivência. Nosso estudo sugere que espécies com sementes achatadas devem ser priorizadas em trabalhos de restauração ecológica florestal e que, na sementeira direta, as sementes quando enterradas aumentam o sucesso da sementeira.

Palavras-chave: sementeira direta, germinação, atributos reprodutivos, espécies arbóreas.

Abstract

Deforestation has increased significantly in the last decades and generated considerable environmental impacts, requiring the establishment of economically viable and effective forest restoration techniques. Direct sowing has now been proposed as an alternative to planting seedlings because of its low cost. However, there are still few works with direct seeding that aim to select species that show highest germination and establishment. Therefore, we carried out a direct sowing study with native tree species of the South Brazilian Semideciduous Seasonal Forest, aim to: (1) determine if reproductive attributes (mass, shape and water content of the seed) interfere with germination; (2) to test the effectiveness of direct seeding in treatments with and without seed cover, buried and non-buried seeds, and (3) establish criteria for selection of species for ecological restoration

using direct seeding. Seeds of 17 species were collected and tested in the laboratory and 12 of them were sowed in the field. The traits seed mass, seed form and water content were related to germination rate, mean germination time and germination speed index for the species tested in the laboratory. In direct seeding, we compared the three treatments 'buried with cover', 'buried without cover', 'not buried with cover' and analysed emergence, establishment and survival. In the laboratory, the flattened seeds presented greater germinability and a higher rate of germination, and the interaction of seed form and mass was significant for germination rate. In direct seeding, buried seedlings with mulch cover presented higher germination rates, but there was no significant effect for establishment and survival. Our study suggests that species with flattened seeds should be prioritized in forest ecological restoration and that, when buried, increase direct seeding success.

Keywords: direct seeding, germination, reproductive traits, tree species.

Apresentação

A presente dissertação foi desenvolvida na forma de um artigo científico, no qual abordamos a germinação de espécies arbóreas, em condições controladas (laboratório) e em campo, sob uma perspectiva de restauração ecológica florestal. Os objetivos foram: (i) avaliar se atributos das sementes influenciam na germinação de sementes de espécies arbóreas nativas, sob condições controladas, e (ii) testar a efetividade da semeadura direta de espécies arbóreas em campo e (iii) estabelecer critérios para seleção de espécies visando à restauração ecológica de áreas degradadas na região fitoecológica da Floresta Estacional Semidecidual (FES), no Rio Grande do Sul.

Na “Introdução geral”, abordamos de forma ampla a situação de degradação de ecossistemas, e em especial, das florestas na região Sul do Brasil, a importância de se desenvolverem técnicas eficazes (baixo custo e alto sucesso) de restauração ecológica e alguns trabalhos com semeadura direta. No capítulo principal da dissertação, apresentamos o artigo "Os atributos das sementes podem contribuir para a efetividade da semeadura direta?", que deverá ser submetido ao periódico *Restoration Ecology*.

Finalmente, no item “Considerações finais”, discutimos alguns aspectos acerca do conhecimento gerado, suas contribuições, perspectivas e sugestões para a pesquisa na área.

Introdução Geral

Nas últimas décadas, a paisagem natural modificou-se drasticamente, e o que eram ecossistemas naturais, hoje em sua maior parte são cidades, indústrias e monoculturas extensas. A humanidade mudou a composição da biosfera e tem transformado os ecossistemas em um ritmo sem precedentes, o qual tem causado danos a todos os seres vivos (Hobbs & Harris 2001). A perda de biodiversidade coloca seriamente em risco o funcionamento do ecossistema (i.e., decomposição de matéria orgânica, ciclagem de nutrientes e retenção de água) e, conseqüentemente, a habilidade da floresta em prover serviços ecossistêmicos. Os serviços ecossistêmicos podem ser *serviços de consumo* como água, madeira, alimentos; *serviços regulatórios* que afetam o clima, polinização, doenças, resíduos e qualidade da água; *serviços culturais* como recreação e benefícios espirituais; e *serviços de suporte* como formação do solo, fotossíntese e ciclagem de nutrientes. Com o aumento da pressão antrópica sobre os ecossistemas florestais, mais do que somente preservar, é preciso restaurá-los (Aerts & Honnay 2011).

Neste contexto, a fim de estabilizar e reverter os processos de degradação é estritamente necessário uma intervenção na qual se possa acelerar e direcionar a sucessão natural (Engel & Parrota 2008). A restauração ecológica é definida como “a ciência, prática e arte de assistir e manejar a recuperação da integridade ecológica dos ecossistemas, incluindo um nível mínimo de biodiversidade e de variabilidade na estrutura e no funcionamento dos processos ecológicos, considerando-se os seus valores ecológicos, econômicos e sociais” (Kageyama *et al.* 2003). Restaurar a integridade de um ecossistema ao seu estado natural parece

praticamente impossível devido às características dinâmicas dos mesmos, porém segundo Young (2000), a premissa básica da restauração é de que muitas forças degradadoras são temporárias e que algumas perdas de habitats e populações são reversíveis.

Para que a restauração ecológica tenha sucesso, devemos desenvolver e buscar técnicas que facilitem os processos sucessionais, aumentando a rapidez, reduzindo os custos e sem necessidade de manutenção pós estabelecimento inicial das plantulas. A técnica mais utilizada para a restauração florestal de áreas degradadas é o plantio de mudas produzidas em viveiros, porém esta é uma técnica cara, trabalhosa e geralmente demorada (Engel & Parrotta 2001; Cole et al. 2011; Tunjai & Elliott 2012; Ceccon et al. 2015). Uma alternativa em expansão é a semeadura direta, a qual é recomendada para áreas extensas, de difícil acesso e com limitação de recursos para a produção e transporte de mudas (Parrotta & Knowles 1999; Sampaio et al. 2007; Bonilla-Moheno & Holl 2010).

Para testar a efetividade da semeadura direta, Ceccon *et al.* (2015) realizaram uma metanálise com base nos dados de 30 artigos e um total de 89 espécies. Os autores constataram que apenas 10,6% desses estudos foram desenvolvidos em zonas subtropicais e apenas 8,9% dos trabalhos referem-se às formações florestais. Dentre as espécies consideradas nesta análise, as taxas de emergência e sobrevivência foram relativamente baixas. O tamanho da semente teve efeito significativo nas porcentagens de germinação e estabelecimento, tendo as sementes de tamanho maior duas vezes mais probabilidade de germinação do que as sementes pequenas, enquanto as sementes médias não tiveram diferenças significativas em relação a estes dois grupos. O sucesso no estabelecimento de

plântulas também foi maior para as sementes grandes. Em um trabalho realizado por Doust *et al.* (2006), foi investigado o efeito de diferentes tratamentos na germinação e estabelecimentos das plântulas em campo, constatando que as maiores porcentagens de germinação e estabelecimento foram encontradas nos tratamentos em que as sementes foram enterradas.

O Rio Grande do Sul abriga uma importante e singular porção de Floresta Estacional Semidecidual (FES), a qual está situada, em sua maior parte, na encosta oriental da mais antiga formação geológica do Estado, a Serra do Sudeste (Rambo, 1956). Nessa região, a FES ocupa uma área superior a 10.000 km², o que corresponde a 0,74% da área do Estado. Porém, 83,6% dessas áreas estão inseridas em ambientes classificados como Antrópico Rural ou Antrópico Urbano, o que evidencia a conversão de expressiva porção dessa formação florestal em uma matriz agrícola/urbana (Luís *et al.* 2009). No entanto, trabalhos que adotam a semeadura direta como técnica para a restauração em áreas de FES são poucos e, em sua maioria, estão limitados às regiões Sudeste e Centro-Oeste do Brasil. No Rio Grande do Sul, Schneider *et al.* (1999) compararam o plantio de mudas e semeadura direta de *Dodonaea viscosa* (Sapindaceae) e não encontraram diferença significativa entre a sobrevivência de plantas do sistema de implementação de mudas e a semeadura direta no campo. Num trabalho realizado na Serra do Sudeste, Velasques & Cardoso (2012) demonstraram que *Parapiptadenia rigida* (Benth.) Brenan, (Fabaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Mimosa scabrella* Benth. (Fabaceae), *Peltophorum dubium* (Spreng.) Taub. (Fabaceae), *Cedrela fissilis* Vell. (Meliaceae) e *Enterolobium contortisiliquum*

(Vell.) Morong. (Fabaceae) mostraram-se eficientes para o uso com esta técnica, tendo essas espécies apresentado porcentagens de sobrevivência $\geq 50\%$.

Na região Centro-Oeste do Brasil, Bechara & Kageyama (2006) demonstraram que *Chamaecrista flexuosa* (L.) Greene (Fabaceae) é recomendada para semeadura direta em restauração de áreas alteradas da FES desde que haja a quebra de dormência das sementes. Santos *et al.* (2012) compararam a emergência, estabelecimento e desenvolvimento de plântulas em campo, em duas áreas com diferentes históricos de degradação (agricultura e pastejo), e demonstraram que a semeadura direta é uma técnica eficiente em ambas as áreas, apesar de ter havido diferenças significativas entre os tratamentos quanto à emergência e sobrevivência das plântulas, com valores mais positivos na área com subsistema agrícola. Silva *et al.* (2015) demonstraram que um sombreamento de 60% pode favorecer o sucesso no estabelecimento das plântulas em campo, bem como controlar o crescimento de gramíneas e herbáceas. Douterlungne *et al.* (2015) demonstraram que a semeadura direta de *Inga vera* Willd. (Fabaceae) apresentou uma resposta eficiente para a restauração ambiental, principalmente pelo seu baixo custo.

Na região sudeste, em duas áreas de preservação permanente, foi testada a semeadura direta com sementes de 16 espécies arbóreas, constatando-se que a técnica é economicamente viável e efetiva para a ocupação inicial de áreas agrícolas em restauração (Isernhagen 2010). Em três fragmentos de FES, também na região sudeste, a técnica de semeadura direta se mostrou viável, obtendo melhores resultados nos tratamentos em que as sementes foram recobertas com palha (Aguirre & Gandolfi, 2012).

Apesar destes estudos o conhecimento acerca da sementeira direta ainda é escasso. Diante disso, o presente estudo teve como objetivos: (1) determinar se os atributos das sementes (massa, forma e conteúdo de água) influenciam na germinação de sementes (em laboratório e em campo); (2) testar a efetividade da sementeira direta em tratamentos com e sem cobertura de palhada, e com sementes enterradas e não enterradas; e (3) estabelecer critérios para a seleção de espécies autóctones para restauração ecológica de áreas degradadas de Floresta Estacional Semidecidual. Nós esperamos que: (1) a massa da semente seja determinante para uma alta germinabilidade, uma vez que sementes maiores apresentam maiores porcentagens de germinação em função da maior quantidade de reserva de nutrientes; (2) que a forma das sementes influencie na germinabilidade, sendo as sementes achatadas aquelas a germinarem mais rapidamente, em função da maior velocidade na absorção de água; (3) que o conteúdo de água da semente influencie na germinação, visto que as sementes com maior conteúdo de água tendem a germinar mais por apresentarem maior longevidade e qualidade e que (4) na sementeira direta haja diferença entre os tratamentos, sendo o tratamento enterrado e com cobertura o que apresente as maiores taxas de emergência, estabelecimento e sobrevivência, visto que sementes enterradas são potencialmente menos susceptíveis à predação e a cobertura de palhada atue reduzindo os extremos de temperatura e aumentando a retenção de água no solo.

ARTICLE: Can seed traits contribute for the success of direct seeding?

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Authors Contributions:

RB, EG e JJ conceived and designed the research; RP performed the experiments; RP analysed the data; RP, AS wrote and edited the manuscript.

Running Head: Seeds traits and direct seeding

Implications:

- Knowing the state of degradation of the area to be worked and applying techniques for soil recovery before sowing should accelerate the restoration process, and facilitate the adoption of direct seeding instead of planting seedlings.
- The growth of seedlings in the field requires control of the surrounding vegetation in the initial stage of establishment to increase survival of seedlings of interest for restoration.
- Flattened seed should be prior on restoration ecology projects.

Abstract

Direct seeding, due to its low cost, has been an interesting alternative but there are a lack of knowledge about the technique and suitable species for restoration ecology. Therefore, we carried out a direct sowing study with native tree species of the South of Brazil, aim to: (1) determine if seed traits (mass, shape and water content of the seed) interfere on germination; (2) to test the effectiveness of direct seeding in treatments with and without mulching, buried and non-buried seeds, and (3) advice for selection of species for ecological restoration. Seeds of 17 species were collected and tested in laboratory and 12 of them were sowed in the field. The traits were related to germinability, mean germination time and germination speed index for the species tested in the laboratory. In direct seeding, emergence, establishment and survival were calculated and the differences among the three treatments were compared: buried+mulching, buried without mulching and unburied+mulching. In laboratory flattened seeds presented greater germinability and the interaction of seed form was significant for speed germination index. In direct seeding, buried+mulching presented higher germination rates, and the difference among treatments only was significant for *Phytolacca dioica*. Our study suggests that species with flattened seeds should be prioritized in forest ecological restoration and that, when buried, increase direct seeding germination.

Keywords: direct seeding, germination, seed traits, tree species.

Introduction

The ecological restoration has been a strategy widely debated and used in forest ecosystems. It consists of a number of technicals developed and improved in order to the recovery of the ecological integrity of ecosystem (Kageyama et al., 2003). The most common method of restoration in degraded areas is planting nursery-raised tree seedlings, however, it is considered costly and requires intense labor (Engel & Parrotta 2001; Cole et al. 2011; Tunjai & Elliott 2012; Ceccon et al. 2015). An alternative to that method is direct seeding, which is recommended to large areas with hard access, or when there is short resource to produce and transport seedlings (Parrotta & Knowles 1999; Sampaio et al. 2007; Bonilla-Moheno & Holl 2010). However, this method present some disadvantages as: the dependence on soil conditions (e. g. lack of nutrients, soil aeration) (Doust et al. 2006), competition with weed and grass (Sun et al. 1995), seed predation (by ants, birds, and small mammals) (Luís et al. 2002), germination difficulties, slow growing (in comparison with nursery-raised tree seedlings) and rise in mortality due to climate conditions (Parrotta & Knowles 1999; Engel & Parrotta 2001; Luís et al. 2002). Even with all these disadvantages, studies have revealed that direct seeding continues to be a productive strategy in ecological forest restoration in large areas (Engel & Parrotta 2001; Guarino & Scariot 2014; Silva & Vieira 2017).

Indeed, some morphological features, known as functional traits, are important to comprehend and predict evolutionary patterns and processes in the plant communities (Pérez-Harguindeguy et al. 2013). In this scenario, seed traits

have an important, but still overlooked role in restoration ecology, since they must determine the capacity of spreading and re-establishment. Germination studies, conducted in the field or laboratory, provide mostly easy-to-obtain data around these traits, and out of this quantification is possible to get important ecological information about the functioning of the communities (Jiménez-Alfaro et al. 2016). Adding data on seed traits in community ecology gives support for the comprehension and functioning of the multi-functional space in plant traits (Laughlin 2014). The traits are morphologically easy to identify and are functionally related to the dispersal processes, therefore, reflecting other properties, such as the persistence in the soil seed bank (Thompson 1993; Moles et al. 2000).

Most studies about reproductive traits refer to seed mass (Shankar 2006), however, its effects in direct seeding are still controversial. Generally, larger seeds present higher content of nutrients, and that could ensure the chances of seedling survival. According to Doust et al. (2006) when buried, larger seeds present higher germination and growing rates than small and medium ones. Seeds of pioneer species, which are mostly small, have been widely used in ecological forest restoration for their natural occurrence in early stages of succession (Sun et al. 1995; Engel & Parrotta 2001; Garcia-Orth & Martinez-Ramos 2008; Balandier et al. 2009). Seedlings grown from small seeds take a long time to develop the first leaves, and their growth is inhibited by shade (Fenner & Thompson 2005). Larger seeds retain the higher amount of resources as reserve material that can be immediately used when necessary (Moles & Westoby 2006). In the other hand, larger seeds have lower absorption of water and more difficult to occupy small

places in the soil. One of the challenges faced by field sown seeds is the exposure to long drought periods until the seedling establishment. Germination can take days or even weeks, in which could occur periods of flood and drought (Fenner & Thompson 2005).

Once combined with seed mass, seed shape seems to affect the probability of seed predation, persistence in the seed bank, dispersal ability, seed production, and establishment success (Liu et al. 2007), confirming its functional character (Campbell et al. 1999). Seeds with round shape showed better penetration in the soil, higher persistence before germination and, therefore, they are often dominant in the seed bank (Yu et al. 2007). Contrary to that, many small-, elongated- or conically-shaped seeds germinate immediately after collection (Grime et al. 1981) because they present shorter distances between the embryo and seed surface, reducing the barrier to moisture and oxygen penetration into the embryo. Furthermore, round-large seeds present difficulties to predators (Cole et al. 2011).

Seed moisture also is a major factor that affects seed quality and longevity. Moisture content and longevity are negatively correlated, except at very low moisture levels (Ellis et al. 1988). The speed germination is related to its moisture content, and moist seeds commonly germinate promptly in open areas, and that is convenient for candidate species of direct seeding (Tunjai 2005).

Another factor that may affect the success of the direct seeding is the way how sowing is performed. According to Doust et al. (2006) seeds, when buried, show higher rates of establishment, but there was no significative difference in the treatments with or without mulching from the site. On the other hand, Silva et al. 2015 state that excessively shaded experiments (covered with manure) decreased

the survival and growth of the seedlings, and there was no difference among the treatments made with or without mulching.

Deforestation has increased significantly in the last decades and generated considerable environmental impacts. In Brazilian Atlantic Forest

In face of the current scenario of deforestation over the past decade, it becomes urgent to implement effective strategies for conservation and restoration of the forest ecosystem. In Brazilian Atlantic Forest, for example, in the period of 2015 to 2016, 29.075 ha (290 Km²) of remaining forest were devastated, an increase of 57.7% in deforestation rates in relation to the previous period (2014-2015) (SOS Mata Atlântica and INPE, 2017). In the southern Brazil (Subtropical Atlantic Forest), specifically, studies concerning direct seeding for ecological forest restoration are still scarce. Therefore, it brings up the importance of researches to improve quantification of costs, to identify more effective strategies, as well as species and/or functional traits related to the success of the forest restoration in these Forests. Facing that, we conducted a direct seeding experiment using native tree species of Atlantic Forest, in order to achieve the following objectives: (1/ observe if seed traits (mass, shape and moisture content/ interfere in the seeds germination rates (in laboratory and in the field/; (2/ test the effectiveness of direct seeding in treatments with or without mulching, buried or unburied; (3) establish criteria for the selection of native species in order to restore degraded forest areas. We expect that: (1) seed mass determine germination rates, once larger seeds must have greater germination rates due to the higher nutrient content; (2) seeds shape influence the speed of germination, considering flattened seeds as the ones with faster germination because of its rapid absorption of water; (3) seed moisture

content influence in germination, for the seeds with greater moisture content present more longevity and quality; (4) the different treatments affect significantly the emergence, survival and establishment of seeds in direct seeding experiment, with seeds buried + mulching emerging, surviving and establishing better than seeds unburied or sown without mulching, considering that buried seeds are potentially resistant to predation, once the mulching works reducing temperature extremes and increasing soil-water- retention.

Materials and Methods

Species studied

We selected 17 species of tree largely distributed within Subtropical Brazilian Atlantic Forest (Table 1). Then, we collected fruits and seeds of each species (at least five matrices). The seeds of each species were mixed and stored in a closed plastic container.

Seed traits

We measured seed traits with 10 seeds per specie (minimum of five matrices) and we calculated the average for each (Pérez-Harguindeguy et al. 2013). To the moisture content, we measured fresh seed mass (with precision four-

digit balance), dried at 60°C for 72 hours in a stove, then we measured the dry mass and calculated the seed moisture content using the ISTA formula (2005):

$$\text{MoistureContent} = \frac{\text{FreshMass} - \text{DryMass}}{\text{FreshMass}} \times 100$$

We obtained seed mass by dry mass of average seeds of the specie for a thousand of seeds, expressed in g. Seed shape was considered as the variance of its three dimensions, i.e. the length, the width and the thickness (breadth) of the dispersule, after each of these values has been divided by the largest of the three values (Thompson, 1993; Pérez-Harguindeguy et al. 2013). Finally, the seeds dimensions were taken with a digital caliper and classified into three categories: round (<0,06), oval (0,06–0,09) and flat (>0,09) (Tunjai & Elliott, 2012).

Pre-germinative treatments

For most of the species, we did not use any technique for breaking dormancy. Although, for some species that had recognized dormancy, such as *E. cristagalli*, *M. coriacea* e *M. lorentziana*, germination was inducted by mechanical scarification with sandpaper on the opposite side of the embryo (Miele et al. 2010; Danielwski et al. 2011; Renata Lucas – pers. comm.). To break the dormancy of *T. micrantha*, the seeds were immersed in water 50°C for five minutes (Floriano 2004). These proceedings were taken in both experiments.

Germination under controlled conditions

The experimental design was completely randomized composed by four replicates with 25 seeds, generating an amount of 100 seeds per species. Initially, for surface disinfection, seeds were immersed in 1% Sodium hypochlorite for 3 minutes and washed with distilled water (BRASIL 2009a). Later, seeds were placed to germination into gerbox® with two layers of filter paper.

The gerbox® was placed into a germination chamber with controlled conditions of light (photoperiod 16h), temperature (25°C) and humidity (80%). The seeds were considered germinated when the radicular had a length of 2 mm (Brasil 2009b) and considered complete when there was no new germination for a period of 15 days. We observed the experiment every 48h, but the first observation was 24h after the beginning. The experiment was conducted at the Laboratory of Ecology of the department of Ecology at Federal University of Rio Grande do Sul and at Laboratory of seeds at EMBRAPA Clima Temperado. We calculated the Mean Germination Time (MGT), the Speed Germination Index (SGI) and the Germinability (G%). The last one was calculated as the percentage of seeds germinated in relation to the total number of seeds. For the MGT we used the following formula (Ferreira & Borghetti 2004):

$$TMG = \sum ni \cdot ti \div \sum ni$$

In which n_i is the number of germinated seeds within a time interval of t_{i-1} e t_i .

For the SGI we used the following formula (Ferreira & Borghetti 2004):

$$IVG = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n}$$

In which G_1, G_2, \dots, G_n is the number of germinated seeds and N_1, N_2, \dots, N_n are the number of days after the sowing.

Direct Seeding

Direct seeding was conducted during February 2016, at Nova Santa Rita de Cássia II Agrarian Reform Settlement, in Nova Santa Rita city, Rio Grande do Sul, Brazil. Until 2004, this area was used for planting conventional rice, which was cultivated with agrochemicals (herbicides and pesticides), according to local residents. Thenceforward, the area is used for planting organic products, seeking to restore soil properties. Considering the soil properties can be the key to success of restoration projects (Heneghan et al. 2008). Based on that, we collected samples of soil and sent them to analysis to obtain information around the physical and chemical parameters for characterization purposes and for the database with similar analyses (Supplementary material 1, Table 1).

From the 17 species collected, only 12 were sown in direct seeding experiment considering the seeds availability of: *A. edulis*, *B. forficata*, *C. fissilis*, *C. montevidense*, *E. cristagalli*, *L. divaricata*, *M. coriacea*, *M. lorentziana*, *P. dioica*, *Q. brasiliensis*, *S. terebinthifolius* e *T. micrantha*. We tested three different treatments i) burial + mulching (BM), ii) unburied + mulching (UM) and, iii) buried without mulching (BW). The mulching used in the experiment was collected from the site of study. The experiment design was totally randomized with ten replicates, and in each treatment 10 seeds per specie were sown, totalizing 100 seeds per specie in each treatment. A plot of ground, with 34,3 m long and 2 m

wide, was made using a tractor with a trowel (Figure 1), and it was divided as illustrated in Figure 2. Each seed was sown 0,5 m apart from the other seeds and from the edges of the plot, and when buried, with a depth of 0,1 m. No method was used to prevent or quantify seed predation. The seeds were considered germinated when visible on the soil or cover. The experiment was followed weekly in the first months, until the germination was finished (considering the process complete when there was no new germination for a period of 15 days), after which it was monitored monthly. The emergence was estimated as the percentage of germinated seeds in relation to the total sown. The establishment is the percentage of seeds germinated in relation to the total of seeded one year after the installation of the experiment. Survival was calculated as the percentage of surviving seedlings one year after the experiment was installed, in relation to the number of sprouts. We also measured the growth of seedlings that survived one year after the experiment was set up.

Statistical analysis

In order to standardize the variable of the seeds, such as MTG, SGI, mass, and shape, were transformed for scores Z. The germination and content variables (A%), which are expressed as a percentage, were submitted to angular transformation or arccosine (arccosine ($\sqrt{\%}$), Zar 1999). All variables were examined for normality and homogeneity of variation using, respectively, the Shapiro-Wilk and Bartlett tests ($P > 0.05$).

The dependent variables of germination under controlled conditions - G%, MTG and SGI - were analyzed using mixed generalized linear models (GLMM, with the Gaussian binding function for normal data). GLMM is an extension of traditional linear models, which estimates the mean of a population depending on a linear predictor using a nonlinear link function, in which the linear predictor contains random effects. This property, combined with the fact that a normal distribution of response variable is not mandatory, and allows a wide range of data analysis (Davidian & Giltinan, 1995).

On the direct seeding experiment, the effects of the treatments on emergence, establishment, and survival were estimated using Two-way ANOVA with Tukey HSD test for post hoc comparisons (Zar 1999). Due to the low number of emergence, we chose not to statistically analyze the effects of seed traits.

All statistical analysis were performed using the R (R Core Development Team 2013) software, with significance level $P > 0.05$.

Results

The seed mass ranged from 0.000023 g (*F. cestrifolia*) to 0.4749 g (*E. cristagalli*), while the seed moisture content ranged from 8% (*F. cestrifolia*) to 67.09% (*C. iguanea*), and seed shape ranged from 0,0093 (*F. cestrifolia* – round) to 0,27 (*C. sylvestris* and *C. fissilis* – flat) (Table 2).

Germination under controlled conditions

Cedrela fissilis had the high G% and a relatively short MGT (Table 2). The average of germinability for flat, oval and round seeds was 51%, 31% and 20.6%, respectively (Figure 3). Seeds with higher G% were *C. fissilis*, *M. coreacea* and *B. forficata* (flattened seeds). Two species did not germinate, *C. iguanea* and *M. lorentziana*, and the species with lower G% were *A. edulis*, *F. cestrifolia* and *D. viscosa* (round seeds) (Figure 2). There was no significant effect of seed traits on germinability (Table 3).

The MTG ranged from 4.8 days (*E. cristagalli*) to 70.4 days (*T. micrantha*) (Table 2) and did not show a significant relation with the seed traits (Table 4). The SGI ranged from 0.40 (*A. edulis*) to 19.41 (*Q. brasiliensis*) (Table 2), and the shape of the seed had a significant effect on the SGI ($p = 0.006$ Table 5).

Direct Seeding

Of the 12 species selected for direct seeding, only three germinated in the field: *A. edulis*, *B. forficata* and *P. dioica*. (Table 6). The effect of the tree different treatments (BM, UM, and BW) was only significant for the emergence of the *P. dioica* ($p \geq 0.0001$) (Table 7). It is had the highest percentage of emergence and survival in BM and BW treatment, respectively (Table 6). Meanwhile, the treatments had a marginal effect on emergency of the *A. edulis* ($p = 0.0595$) (Table 7), which had the greatest percentage in BM treatment, as well as for establishment. Moreover, *A. edulis* had the highest percentage of survival among the tree species for all treatments (Table 6). In relation to *B. forficata*, the highest

percentage of emergence and establishment occurs in BM and BW treatment, respectively (Table 6), while the survival was higher in UM treatment (Table 6).

Discussion

In this study we aimed to evaluate the relation between seed traits and the success of the tree species germination under controlled conditions, as well as the effectiveness of direct seeding.

Germination under controlled conditions

The results of germination under controlled conditions showed that seed shape was determinant to SGI. These results corroborate with our initial expectative that flattened seeds germinated faster than oval and round. Liu et al. (2007) demonstrated that flattened seeds tend to germinate immediately after dispersion and present higher percentages of germination. Faster germination decreases susceptibility to predation and increases seedlings survival (Lamb 2011). However, only this trait does not guarantee the establishment of seedlings on the field. In addition to that, fast growth allows seedlings to compete with the surrounding vegetation, which is an important capacity, required for the success of direct seeding (Engel & Parrotta 2001; Doust 2011). In spite of the fast germination in flattened seeds, they tend to have a low capacity for stress tolerance (Moles & Westoby 2006; Muller-Landau 2010) and pathogens (Guarino & Scariot 2014). According to Yu et al. (2007), round seeds penetrate more easily into the soil and

this decreases the chances of predation and susceptibility to desiccation. So considering the persistence of round seeds into soil seed bank it could be a *trade off* in relation to germination, explaining the high germinability of flattened seeds, followed by oval and round, in this study.

Our results demonstrated that seed mass had no relation with germination parameters, although it has been described in current studies that it is an important trait which influences seed germination (Turnbull et al. 1999; Moles et al. 2005; Doust et al. 2006; Lamb 2011). The advantage of larger seeds seems to be related to a higher capability to support stress because the high nutrient content that allows them to survive on extreme conditions such as drought and shadow, although these advantages are not the same under others conditions (Coomes & Grubb 2003; Muller-Landau 2010).

Seed moisture content had no significant effects on G%, MTG and SGI, although it has been cited as the main factor that affects seed longevity and the quality of seeds (Tunjai & Elliott 2012), however, in some cases it has no relation with germination (Sautu et al. 2006).

Direct Seeding

Considering the total of sown species, only 25% emerged (3 species). This situation is frequent in studies of restoration ecology, in which the most of the species sowed did not emerge and just a few presented high percentages of establishment (Engel & Parrotta 2001; Doust et al. 2006).

In relation to the differences among treatments, our results demonstrated that only emergence of *P. dioica* was affected significantly. No significant difference was found for the other parameters. Notwithstanding, the buried + mulching treatment presented the highest emergence for all species, corroborating in part what we expected. Similar studies have shown that mulching decreases extreme temperatures and increases soil water retention (Truax & Gagnon 1993; Dostálek et al. 2007) and probably benefits seedlings in the the field (Silva et al. 2015). Another study demonstrated that *S. terebinthifolius* had more survival percentage when seeds were buried with mulching (Hüller & Peske 2011). The establishment and survival were critical in our study that may be due to the grass growth in the experimental units, requiring constant management to suppress competing vegetation (Silva et al. 2015). The low success of direct seeding in the experiment could be related to soil conditions which are dystrophic, alic and has a low retention humidity (Tomé 1997; see Supplementary Material Table 8).

In this study, we did not evaluate underground and aerial growth, but it is known that plants allocate resources differently in their parts during their growth, this being one of the key factors for its development and survival (Weiner 2004; Fenner & Thompson 2005). Studies in this direction are needed in order to give better subsidies to select the species that best adapt to direct seeding.

Taking into account the species we broke dormancy, none of them emerged in the field. This technic is recommended to accelerate germination, but it increases the probability of mass death if it is succeeded by a dry period and/or attacks by herbivores (Silva et al. 2015). Further studies are needed to evaluate the effectiveness of seed dormancy breaking in direct seeding.

Due to the lack of data, it was not possible to evaluate the effects of seed traits on emergence, establishment, survival, and growth in the field. On recent direct seeding study, Silva & Vieira (2017) demonstrated that round seeds show no difference on establishment when buried. On the other hand, flattened seeds were negatively affected and had lower emergence when cover with mulching.

In order to overcome the limitation of emergence, establishment and survival of the seedlings in the field, the technique of direct seeding should be improved taking into account the depth of planting, seed viability, reduction of the incidence of pathogens and herbivores, reduction of exposure to high temperatures in open areas, to avoid desiccation of seeds, and especially the control of competing herbs (Silva et al., 2015).

Conclusion

The present study aimed to investigate the relation between seed traits and germination of tree species (in lab) and to test the effectiveness of distinct methods for direct seeding. In germination under controlled conditions we verified that flattened seeds germinated faster than oval and round ones. In direct seeding experiment, the distinct treatments only affect the emergence of *P. dioica*. Notwithstanding, when seeds were buried and covered with mulching, they presented the highest percentage of emergence for all species.

As the shape of the seed was determinant for rapid germination, we suggest that this trait should be considered in the process of selection of species to be used for forest ecological restoration. Due to the low emergence and survival of the seedlings in the field, an alternative could be the combination of direct seeding with

the planting of seedlings, which can increase the success of the ecological restoration.

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Table 1. List of species collected for the study and their families

Specie	Family
<i>Allophylus edulis</i> (A.St.-Hil. et al.) Radlk.	Sapindaceae
<i>Bauhinia forficata</i> Link	Fabaceae
<i>Casearia sylvestris</i> Sw.	Salicaceae
<i>Cedrela fissilis</i> Vell.	Meliaceae
<i>Celtis iguanaea</i> (Jacq.) Sarg.	Cannabaceae
<i>Citharexylum montevidense</i> (Spreng.) Moldenke	Verbenaceae
<i>Dasyphyllum tomentosum</i> (Spreng.) Cabrera	Asteraceae
<i>Dodonaea viscosa</i> Jacq.	Sapindaceae
<i>Erythrina cristagalli</i> L.	Fabaceae
<i>Ficus cestrifolia</i> Schott	Moraceae
<i>Luehea divaricata</i> Mart.	Malvaceae
<i>Myrsine coriacea</i> (Sw.) R.Br.	Primulaceae
<i>Myrsine lorentziana</i> (Mez) Arechav.	Primulaceae
<i>Phytolacca dioica</i> L.	Phytolaccaceae
<i>Quillaja brasiliensis</i> (A.St.-Hil. & Tul.) Mart.	Quillajaceae
<i>Schinus terebinthifolius</i> Raddi	Anacardiaceae
<i>Trema micrantha</i> (L.) Blume	Cannabaceae

Table 2. Germinability (G%), Mean Time to Germination (MTG), Speed Germination Index (SGI), mass (g), shape, moisture content and format for 17 species from South of Brazil.

Specie	G%	MTG	SGI	Mass	Shape	Water	Format
<i>Allophyllus.edulis</i>	3	12,75	0,4005	30,7	0,0447	24,2	Round
<i>Bauhinia.forficata</i>	79	27,0521	9,3599	128,3829	0,1356	13,07	Flat
<i>Casearia.sylvestris</i>	55	40,4953	5,8488	1,3	0,27	10,34	Flat
<i>Cedrela.fissilis</i>	80	8,661	11,1813	17,934	0,2733	14,18	Flat
<i>Celtis.iguanea</i>	0	0	0	59,15	0,0445	67,09	Round
<i>Citharexylum.montevicense</i>	18	55,7	1,8665	37,3	0,077	19,18	Oval
<i>Dasyphyllum.tomentosum</i>	26	10,5985	6,504	0,7785	0,2563	19,15	Flat
<i>Dodonea.viscosa</i>	17	34,9458	1,6724	9,3	0,2634	51,81	Flat
<i>Erythrina.cristagali</i>	24	4,7667	3,8637	474,9	0,0902	10,09	Oval
<i>Ficus.cestrifolia</i>	10	14,7333	0,4312	0,023	0,0093	8	Round
<i>Luehea.divaricata</i>	38	20,5643	7,4684	9,2962	0,2501	13,96	Flat
<i>Myrsine coreacea</i>	80	60	1,7131	5,2	0,0002	46,94	Round
<i>Myrsine lorentziana</i>	0	0	0	3,5	0,0012	48,86	Round
<i>Phytolacca.dioica</i>	67	16,4359	6,9597	2,5	0,2538	65,75	Flat
<i>Quillaja.brasiliensis</i>	73	10,262	19,4136	5,5362	0,2532	11,48	Flat
<i>Schinus.terebinthifolius</i>	44	13,339	5,2418	13,2325	0,0669	28,03	Oval
<i>Trema micrantha</i>	30	70,4015	0,7352	12,555	0,0168	14,39	Round

Table 3 : Resume of Mixed generalized linear model for germinability. Degrees of freedom = 0,05.

Variable	Value	Std. Error	DP	t-value	p-value
intercept	0.8095543	0.2300154	13	3.5196	0.0038
Mass	0.0142088	0.0913642	13	-0.156	0.8788
Shape	0.1646727	0.0894922	13	1.8401	0.0887
Water Content	0.3610741	0.4027414	13	-0.897	0.3863

Table 4: Resume of Mixed generalized linear model for mean time to germination. Degrees of freedom = 0,05.

Variable	Value	Std. Error	DP	t-value	p-value
intercept	0.4992	0.6829	13	0.731	0.4778
Mass	-0.305	0.2713	13	-1.1242	0.2812
Shape	-0.228	0.2657	13	-0.8578	0.4065
Water Content	-0.94	1.1958	13	-0.7862	0.4458

Table 5: Resume of Mixed generalized linear model for speed germination index. Degrees of freedom = 0,05.

Variable	Value	Std. Error	DP	t-value	p-value
Intercept	0.6765	0.4979	13	1.3587	0.1974
Mass	-0.011	0.1978	13	-0.055	0.9574
Shape	0.6363	0.1937	13	3.2844	0.006
Water Content	-1.274	0.8719	13	-1.461	0.1677

Table 6. Emergence, establishment, survival and main growth in each treatment for species germinated in direct seeding experiment. B+M (buried+mulching), UM (unburied+mulching) and BW (buried without mulching). Highers values are in bold.

Specie	Treatment	Emergence %	Establishment %	Survival %	Main Growth (cm)
<i>Allophylus edulis</i>	BM	22	14	63,63	4,92
	UM	8	7	87,5	5,14
	BW	13	12	92,3	4,79
<i>Bauhinia forficata</i>	BM	24	3	15,78	8,66
	UM	12	9	75	7,66
	BW	19	12	50	6,12
<i>Phytolacca dioica</i>	BM	52	1	1,92	2,5
	UM	13	0	0	0
	BW	45	1	2,22	2

Table 7. Values of *P* and *F* for ANOVA test. Emergence, establishment, survival and main growth in each treatment for species in direct seeding experiment. Higher values are in bold.

Specie	Emergence		Establishmente		Survival		Growth	
	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>
<i>Allophylus edulis</i>	0.0595	3.138	0.391	0.972	0.649	0.439	0.456	0.808
<i>Bauhinia forficata</i>	0.681	0.39	0.391	1.058	0.534	0.641	0.412	0.915
<i>Phytolacca dioica</i>	2.97e-06	21.16	0.612	0.5	0.584	0.549	0.61	0.503



Figure 1: Plots of direct seeding experiment at Nova Santa Rita de Cássia II Agrarian Reform Settlement, in Nova Santa Rita city, Rio Grande do Sul, Brazil.

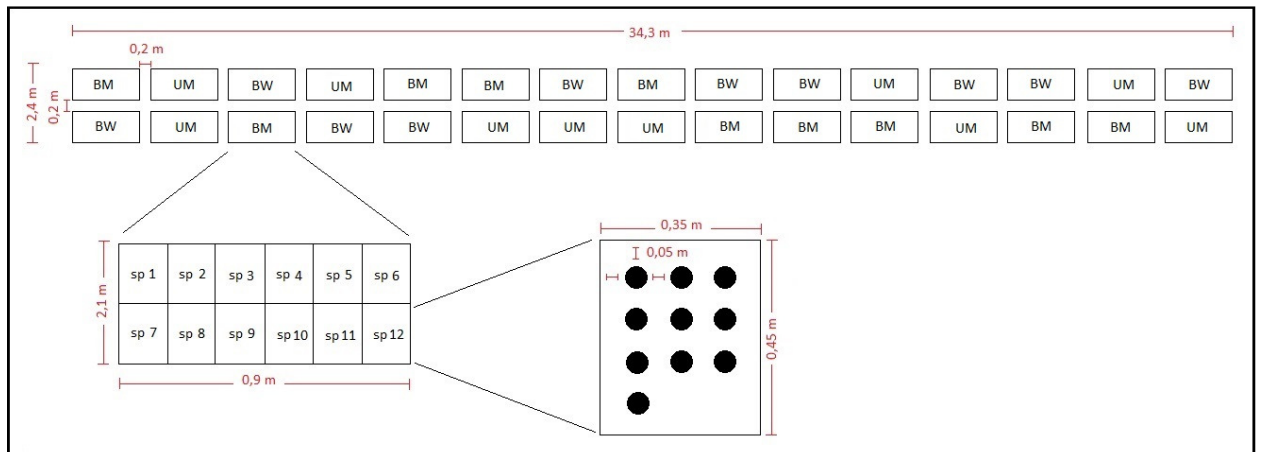


Figure 2: Design of direct seeding experiment. BM (buried+mulching), UM (unburied+mulching) and BW (buried without mulching).

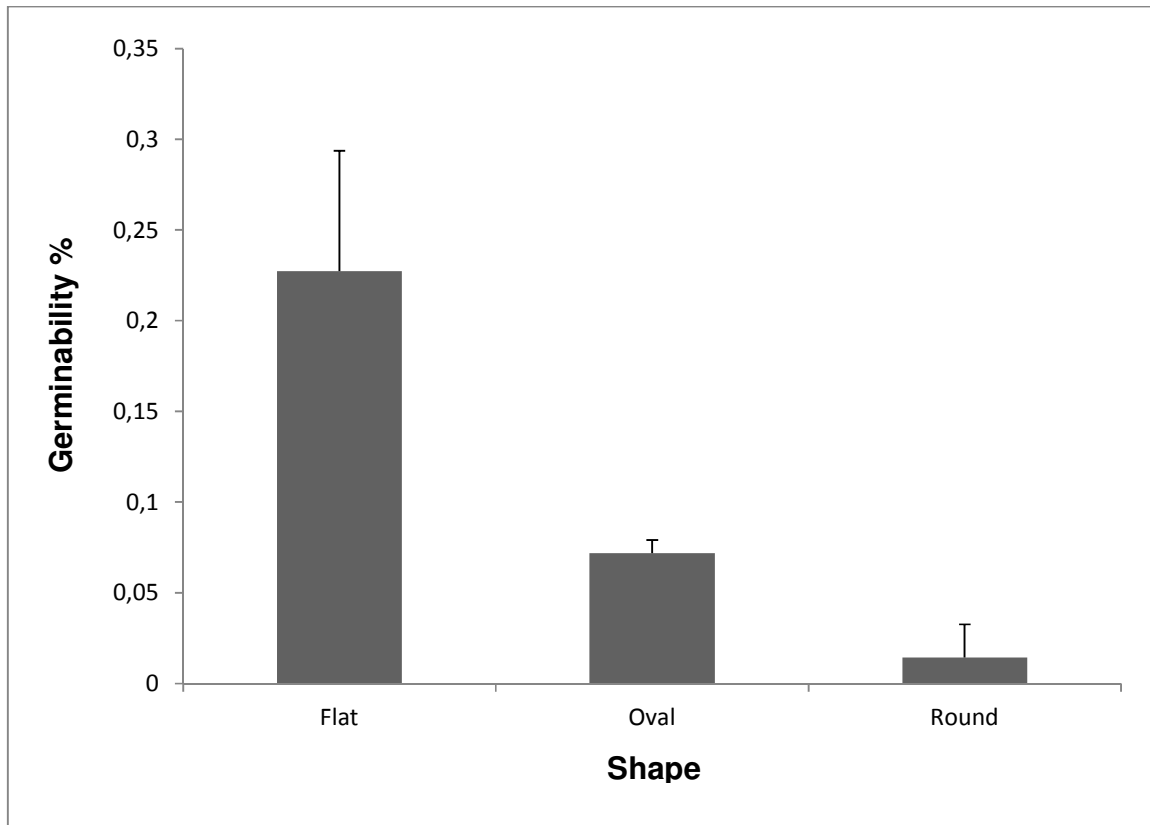


Figura 3: Shape and germinability of 17 species from souht of Brazil.

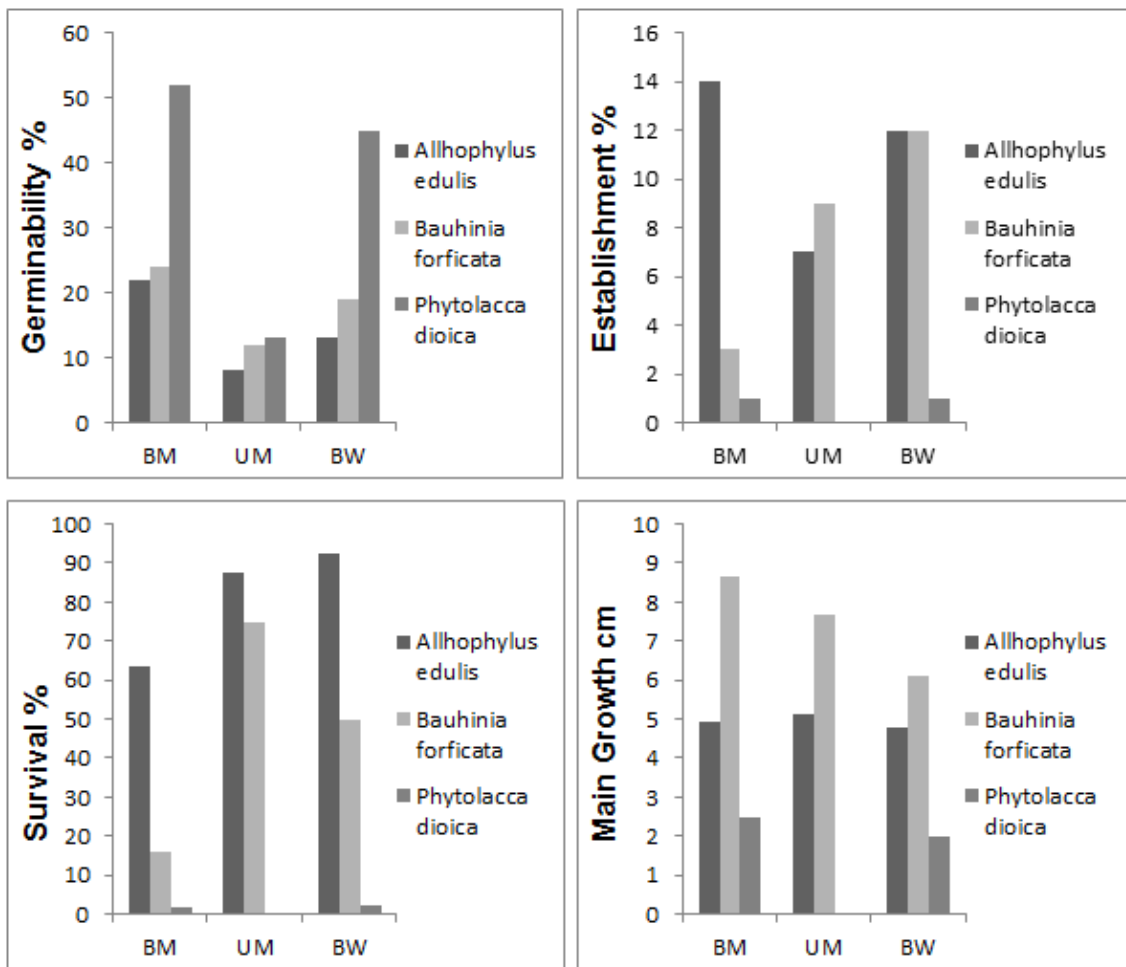


Figure 4: Emergence, establishment, survival and main growth for species in each treatment of direct seeding experiment: BM (burrial+mulching), UM (Unbirried+mulching) end BW (buried without mulching).

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Supplementary Material

S1 Table 1. Physical and chemical parameters analyzed from soil samples at the site of direct seedling at Nova Santa Rita (RS).

pH água 1:1	5,2	K (mg/dm ³)	50
Índice SMP	6,6	CTC efetiva	1,6
H+Al (cmol c/dm ³)	2,3	CTC pH 7	3,7
Al (cmol c/dm ³)	0,2	B (mg/dm ³)	0,3
Ca (cmol c/dm ³)	0,9	Cu (mg/dm ³)	0,9
Mg (cmol c/dm ³)	0,4	Zn (mg/dm ³)	2,8
K (cmol c/dm ³)	0,1	Mn (mg/dm ³)	25,8
Saturação Al (%)	12,5	Fe (mg/dm ³)	1,9
Saturação Bases (%)	38	Ca/Mg	2,2
Argila (%)	11	Ca/K	9,0
M.O. (%)	1,2	Mg/K	4,0
P (mg/dm ³)	4,2	(Ca+Mg)/K	13,0

Considerações finais

Devido à alta diversidade das florestas tropicais, a seleção de espécies arbóreas a serem selecionadas para projetos de restauração não é uma tarefa fácil. Os estágios iniciais de germinação e crescimento (sementes e plântulas pequenas)

são mais vulneráveis às condições ambientais do que as mudas produzidas em viveiros. A semeadura direta apresenta um desafio maior do que o plantio de mudas, portanto, a exclusão de espécies que apresentam maiores probabilidades de falhar na semeadura direta deve aumentar as chances de sucesso das técnicas de restauração florestal ecológica. As espécies que trabalhamos são relativamente fáceis de serem encontradas (espécies de ocorrência frequente), sendo muito importante levar em consideração a facilidade de se encontrar indivíduos para a coleta de frutos e/ou sementes, para que a técnica seja de fato econômica e viável.

Experimentações em campo não são trabalhos fáceis, principalmente aqueles que envolvem aspectos ecológicos. Existem diversas variáveis (por exemplo, variáveis climáticas, predação e competição) que são difíceis de serem controladas. Levar em consideração essas dificuldades em campo, bem como o planejamento do estudo (tamanho da área, equipe e tempo disponível) pode auxiliar no sucesso da experimentação em campo e reduzir os custos e tempo de experimento.

O presente trabalho traz contribuições no sentido de conhecer a germinação de espécies arbóreas da Floresta Estacional Semidecidual e estabelecer critérios de seleção de espécies para a restauração ecológica florestal desta fitofisionomia. Os resultados encontrados sugerem que alguns atributos devem ser levados em consideração na escolha das espécies a se trabalhar. Embora o número de espécies estudadas seja relativamente baixo, este trabalho abre perspectivas para que outros estudos considerem os atributos das espécies a serem trabalhados. Unir os conhecimentos acerca da ecologia funcional pode levar ao sucesso em trabalhos de restauração ecológica.

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