Research Article
doi: 10.1590/01047760202329013212
vol(29), 2023

CERNE

Strategies for optimizing the enrichment direct sowing: inoculation with *Trichoderma* spp. and use of a hydrogel

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SILVICULTURE

ABSTRACT

Background: Direct sowing technique is considered a promising tool for reducing costs and increasing species diversity in restoration projects. However, several factors can limit its success, making it necessary for methodological adjustments to be made in order to maximize the emergence, establishment and initial growth of plants. This study aimed to evaluate the influence of the use of fungus *Trichoderma asperelloides* and hydrogel in the enrichment direct sowing as a strategy to enrich the area in the initial restoration process. The forest species studied were *Balfourodendron riedelianum*, *Cedrela fissilis*, *Luehea divaricata*, *Enterolobium contortisiliquum* and *Parapiptadenia rigida*. The experiment was conducted in a factorial design testing absence or presence of the *T. asperelloides* and absence or presence of hydrogel. Two experiments were established: in the nursery, we evaluations of the emergence of the seedlings were carried out; in the field, we evaluated emergence, survival, height and stem diameter.

Results: Under nursery conditions, inoculation and the use of hydrogel did not influence the emergence of species tested. Direct sowing proved to be a promising technique for the enrichment of young restoration plantations in subtropical regions with *E. contortisiliquum*, *P. rigida* and *C. fissilis. B. riedelianum* and *L. divaricata* seeds did not germinate in the field.

Conclusion: The use of *T. asperelloides* is an efficient strategy to enhance the results of direct sowing, capable of providing higher rates of emergence for *E. contortisiliqumm* and *P. rigida* and maximizing the growth in height of *E. contortisiliqumm*. Under high pluviometric intensity, the use of the hydrogel does not influence the emergence and morphological attributes of the species studied.

Key words: Atlantic Forest Biome; Beneficial microorganisms; Silvicultural practices; Water-retaining polymer.

HIGHLIGHTS

We studied the effect of inoculation and hydrogel hydration of seeds of tree species.
Inoculation with *Trichoderma asperelloides* increase emergence, survival and growth.
Hydrogel had no effect on emergence and seedling survival.
Direct sowing is a promising technique for the enrichment of areas in subtropical regions.

GRIEBELER, A. M.; ARAUJO, M. M.; STEFFEN, G. P. K.; TURCHETTO, F.; RORATO, D. G.; BARBOSA, F. M.; BERGHETTI, A. L. P.; GASPARIN, E.; AIMI, S. C.; COSTELLA, C. Strategies for optimizing the enrichment direct sowing: inoculation with *Trichoderma* spp. and use of a hydrogel. 2023. CERNE, v.29, e-103212, doi: 10.1590/01047760202329013212.

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Received: January, 6/2023 Accepted: June, 13/2023

CERNE
ISSN 0104-7760
UNIVERSIDADE FEDERAL DE LAVRAS

INTRODUCTION

Direct sowing technique is considered a promising tool for reducing costs and increasing species diversity in restoration projects. This technique can be used both for the introduction of species in areas without vegetation cover (Guerra et al., 2020; Jesus et al., 2017; Rodrigues et al. 2019) and for the enrichment of secondary forests and areas under restoration, where there is already a certain amount of canopy coverage (Atondo-Bueno et al., 2018; Cole et al., 2011; Rodrigues et al., 2019). However, several factors limit the success of direct sowing in restoration projects, including low emergence rate, lack of adequate methods to overcome dormancy, sensitivity to desiccation, predation of seeds and seedlings, competition with invasive plants, low survival and development of emerged plants (Ceccon et al., 2016; Meli et al., 2018; Souza and Engel, 2018; Souza et al., 2020). There is still a lack of information on land preparation methods aimed at improving conditions and creating favorable microsites (Atondo-bueno et al., 2016; Palma; Laurance, 2015; Sangsupan et al., 2018). Thus, to adapt establishment and management methods to overcome these adverse factors might be an alternative to improve the success of direct tree seeding systems (Souza et al., 2021).

One potential strategy that has been used in forest nurseries is the use of beneficial microorganisms to increase emergence rates and initial growth of forest tree species (Amaral et al., 2017; Junges et al., 2016; Machado et al., 2015; Peccatti et al., 2020). The species of the genus *Trichoderma* are among the most studied fungi because they act as agents in the biological control of phytopathogens and in increasing the rates of seed germination and plant growth (Altomare et al., 1999; Griebeler et al., 2021). According to Machado et al. (2012), the ability of species of the genus *Trichoderma* to stimulate the germination and growth may be related to the production of plant phytoregulators and to the solubilization of nutrients in the rhizosphere, allowing greater absorption and translocation by the plant. Despite the beneficial contribution of *Trichoderma* species to the production of forest seedlings, there are no studies available evaluating their effects on restoration projects through direct sowing.

Another important factor in direct sowing is the maintenance of soil moisture, since water restriction can prevent or delay germination and seedling development. The presence of moisture is essential for the resumption of metabolism and initiation of cellular events, triggering the germinative process, which follows a three-stage pattern of hydration, identified by the stages of water absorption, preparation for growth and the protrusion of the radicle (Bewley et al., 2013). In order to ensure favorable humidity for germination and seedling establishment in the initial stage, the hydrogel (water-retaining polymer) incorporated into the soil can be used at the time of sowing. This polymer have the capacity to absorb water during periods of water availability and release it to the roots of plants when there is a deficit in the soil (Leciejewski, 2009). Previous studies have already shown positive effects of hydrogel on tree nursery-grown seedling development (Griebeler et al., 2022; Turchetto et al., 2020). However, studies that evaluate its effectiveness in direct sowing of forest species are scarce (Souza, 2022).

Inoculation of *Trichoderma* spp. and hydrogel application already have been tested in forestry and agriculture to promote increased germination and growth of seedlings in nursey, maximize water availability, and improve microclimatic and edaphic conditions in the field (Griebeler et al., 2022; Racic et al., 2018; Sankar et al., 2019), however, its potential beneficial effects on tree species performances in direct seeding techniques for forest restoration have not been studied yet. Thus, the study was carried out to evaluate the influence of the use of *Trichoderma asperelloides* and hydrogel alone, or in combination, in the direct sowing of five native forest tree species as a strategy for the enrichment of the area in the initial restoration process, in the extreme south of the Atlantic Forest Biome. We sought to answer the following questions: a) for which species is direct sowing a suitable technique to enrich areas undergoing restoration? b) does the inoculation with *Trichoderma asperelloides* provide greater emergence and initial growth of seedlings of the forest species tested? c) does the use of hydrogel favor the emergence and initial establishment of seedlings in direct sowing?

MATERIAL AND METHODS

Study area

The study was carried out in an area of riparian forest located in Santa Maria, Rio Grande do Sul, Brazil, extreme south of the Atlantic Forest Biome (29º 20′ 36.25″ S and 53º 26′ 17.84″ W) (Figure 1).

The soils of the region are classified as Litolic Neosol and Regolitic Neosol (Embrapa, 2013) and vegetation is inserted in the phytogeographic region of Subtropical Seasonal Forest (Schumacher et al., 2011). The local climate is Cfa (humid subtropical) as per the Köppen climate classification (Alvares et al., 2013). The monthly rainfall and the minimum and maximum average monthly temperatures recorded during the period of the study (September 2017 to September 2018) are shown in Figure 2. The data were obtained from the meteorological station at the Universidade Federal de Santa Maria. The light incident in the sub-forest between the initial planting rows was obtained with a light meter (Mlm1011 Minipa) on a sunny day, between 11:00 am and 2:00 pm, and was of approximately 65.0%.

The experimental area for more than 30 years, land use across the property has consisted of intensive agricultural production, with short fallow periods. In 2013, the experimental area was isolated and tree seedlings, in order to start the recovery of the riparian area. The species used were: *Casearia sylvestris* Sw. *Cupania vernalis* Cambess. *Eugenia involucrata* DC. *Inga vera* Willd. *Parapiptadenia rigida* (Benth.) Brenan and *Schinus terebinthifolius* Raddi (Rorato, 2017). However, four years after planting, the site still had an undeveloped understory, with limitations in self-regeneration due to the difficulty in the arrival of propagules, because of its isolation in the landscape of the region (Figure 1).

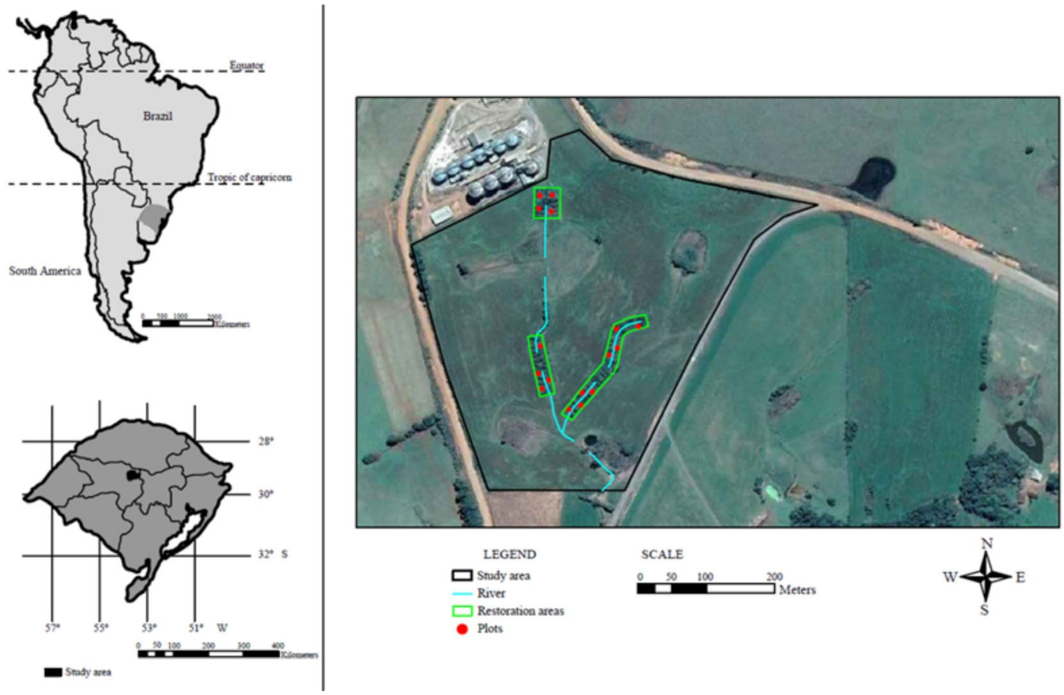

Figure 1. Location of the study area in the extreme south of the Atlantic Forest Biome.

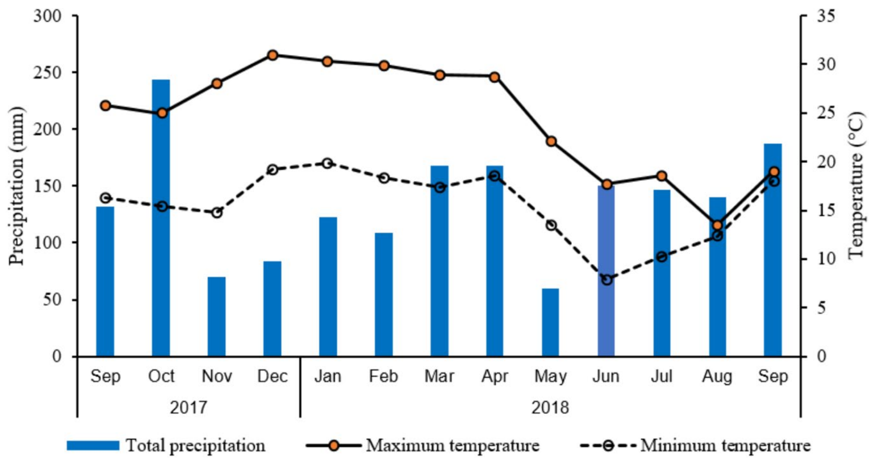

Figure 2. Mean monthly minimum and maximum temperatures (ºC) and monthly precipitation (mm) recorded during the research.

Study species

Five native tree species were selected based on their ecophysiological and silvicultural characteristics, which included: importance in the forest typology of the region; fruiting season and seed availability; as well as their potential to restoration and enrichment projects, observed in preliminary evaluations carried out in plantings conducted in the extreme south of the Atlantic Forest Biome (Table 1). The seeds were collected months (at most) or immediately prior to sowing, from mother trees located in forest fragments in the central region of Rio Grande do Sul, and stored in Kraft paper drums, in a cold and humid chamber until the establishment of the experiments. The dormancy of the seeds of *B. riedelianum* and *E. contortisiliquum* was overcome (Table 1).

Experimental design, establishment and maintenance of the experiment in the field

The experiment was established in September 2017, between the rows of the existing planting, in a randomized block design with four replications. The treatments were distributed in a factorial design, considering the seed inoculation (with *Trichoderma asperelloides* and control

- without inoculation) and the hydration with hydrogel (presence or absence). Each of the 16 plots received 20 pits, four for each species, distributed in two rows with spacing of 1 m x 4 m. The sequence of species in each row was randomized. For the treatment with seed inoculation, a solution containing spores of the isolate *Trichoderma asperelloides* was used. Detailed of isolate and description of the procedures performed for preparing the inoculum solution can be seen in Griebeler et al. (2021).

The planting rows between the existing plant community were cleaned, by manual weeding and pit opening with the aid of a manual post hole digger (15 cm in diameter x 15 cm in depth). Then, in the treatments with the presence of the water-retaining polymer, we added 1.0 L of hydrated hydrogel per pit (4 g L^{-1}), while in the control, we applied 1.0 L of water. The hydrogel used is a mixed product of acrylamide copolymer (C_3H_5NO) and potassium acrylate ($K_2S_2O_8$). The polymer were incorporated in the pit and covered with a layer of soil, thus preparing the pits for sowing. Seed inoculation was carried out before sowing, by immersing for five minutes, in the solution of *T. asperelloides* or in distilled water, for treatment with inoculation and control, respectively.

Sowing was carried out manually at a depth of twice the diameter of the seeds, using five propagules of the same species per pit. The seeds were covered with a thin layer of soil and flagged with bamboo stakes to facilitate monitoring. We did not perform thinning when more than one seedling emerged per point, in order to allow intraspecific competition and natural selection to act.

Whenever the occurrence of competition with invasive plants was observed, we performed the manual weeding in a radius of 30 cm, with the aid of a hoe, taking care not to displace seeds and seedlings. When necessary, we controlled the leaf-cutting ants with Sulfuramid-based granular baits. We provided mineral cover fertilization to the seedlings at 60 days after sowing, using 24 g of urea, 40 g of simple superphosphate and 72 g of potassium chloride. The fertilizers were applied in side pits 15 cm away from the central seedlings.

Field mensurements

We monitored the field experiment for one year after sowing. Seedling emergence assessments were made weekly during the first month of evaluation, and monthly until 150 days. Seedlings with visible cotyledons were classified as emerged, with the results expressed as a percentage and calculated according to the total number of seeds sown by species in each treatment. To assess the performance of the species in terms of their potential for establishment, we evaluated survival, based on the percentage of living seedlings at 12 months after sowing (m.a.s) in relation to the percentage of seedlings that emerged. We also analyzed the suitability of species for direct sowing, and it was defined according to their mean survival rate: suitable species (10.0 % or more), unsuitable species (<10.0 %) and failed species (not established) (Souza and Engel, 2018). At the end of the experiment, we measured the height of the plants between the soil surface and the apical bud (cm) to evaluate their initial growth. In addition, we measured the stem diameter, using a digital caliper (mm).

Greenhouse emergence test

In the same week of the implantation of the study in a field, we established an emergence test in a nursery, in order to verify the viability of the seeds used. For this, we conducted an experiment in a greenhouse, in a completely randomized design with three replications per species,

Table 1: Selected species, dormancy treatments applied to the seeds and their ecological and silvicultural characteristics.

Scientific name	Thousand-seed weight (g)	Pre-germination treatments	SG	Species characteristics
Balfourodendron riedelianum (Engl.) Engl [Rutaceae]	361.40	Cold water immersion 10 °C for 48 h[1]	LS[3]	Indicated for planting in degraded areas and reforestation for commercial purposes. Valuable wood used for luxury furniture and construction
Cedrela fissilis Vellozo [Meliaceae]	30.79	No treatment	LS[3]	Recommended for restoration and commercial plantings and urban afforestation. Wood is valuable in construction and furniture manufacturing. It has medicinal properties
Luehea divaricata Mart. & Zucc [Malvaceae]	4.18	No treatment	IS[3]	Indicated for restoration of riparian forests and mixed plantings. Wood with good workability indicated for making turned furniture.
Enterolobium contortisiliquum (Vell.) Morong [Fabaceae]	747.60	Manual scarification with sandpaper[2]	PI[3]	Used in the recovery of degraded areas and urban afforestation, it has rapid growth. Wood used in civil and naval construction. They have medicinal uses.
Parapiptadenia rigida (Benth.) Brenan [Fabaceae]	27.70	No treatment	IS[3]	Recommended for restoration and commercial plantings and urban afforestation. Dense wood used in civil and rural construction. It has medicinal propertie.

Where: SG: successional group; PI pioneer; IS: initial secondary; LS: late secondary. [1]Gomes et al. (2016); [2]Brasil (2013); [3]Carvalho (2003).

containing twenty containers each plot. The treatments evaluated were the same tested in the field. The containers consisted of polypropylene tubes with a capacity of 180 cm^3. The tubes were filled with Sphagnum peat-most substrate, vermiculite and carbonized rice husk, we added controlled release NPK fertilizer (15:09:12) in the dosage of 5 g L^{-1} of substrate and hydrogel (4 g L^{-1}). After the seed inoculation procedure with *T. asperelloides* (detailed in the installation of the field experiment), sowing was carried, using three seeds per container. The containers remained in the greenhouse and were irrigated by micro sprinklers (8 mm day^{-1}). The emergence counting was carried out every seven days, from the date of establishment of the test, up to 60 days, through visual observation and record of emerged seedlings.

Statistical analysis

The data were submitted to the analysis of the assumptions of normality of residuals and homogeneity of variance by the Shapiro-Wilk and Bartlett test, respectively. Subsequently, the fixed effect analysis of variance (Factorial ANOVA) was performed, with a 2 x 2 factorial design (inoculation x hydration). The means were compared using the Student's t-test (α 5%), with the aid of the SISVAR statistical software (Ferreira, 2014). The species that did not germinate were not considered in the analyses.

RESULTS

Seedlings emergence in greenhouse conditions

We did not observe any interaction or significant difference between the study factors (inoculation x hydration) for the percentage of emergence ($p>0.05$). *Enterolobium contortisiliquum*, *Parapiptadenia rigida* and *Cedrela fissilis*, had the highest rates of emergence, with values above 97.0 %. *Luehea divaricata* and *Balfourodendron riedelianum* demonstrated low seed germination potential, with 38.8 % and 32.1 %, respectively (Figure 3).

Seedling emergence and survival in the field

The emergence of seedlings in the field, 150 days after sowing differed significantly between species, with the overall mean of the experiment being 11.2 %. There was no interaction between the study factors, but expressed a significant effect of the inoculation of the seeds with *T. asperelloides* for *E. contortisiliquum* (p = 0.0075) and *P. rigida* (p = 0.0171), and the percentage of emergence was about 102.0 % and 303% higher, respectively, with the use of the fungus compared to the control (Figure 4). The emergence was null for the species *L. divaricata* and *B. riedelianum* in all treatments. There was no significant influence ($p>$ 0.05) of the use of hydrogel on the variables or species evaluated.

When seed inoculation was carried out, we observed the peak of accumulated emergence at 21 d.a.s for the species *E. contortisiliquum* (a) and *C. fissilis* (c), and at 14 days after sowing for *P. rigida* (b), stabilizing after 60 days for *E. contortisiliquum* and *P. rigida* and after 90 days for *C. fissilis* (Figure 5).

The analysis of seedling survival was not affected by inoculation or hydration ($p>$ 0.05). We observed a high survival rate of the seedlings that emerged, with mean survival rates above 87.0 %. Although the seedling emergence in the field was low for *P. rigida* and *C. fissilis*, survival was high for these species (Figure 6).

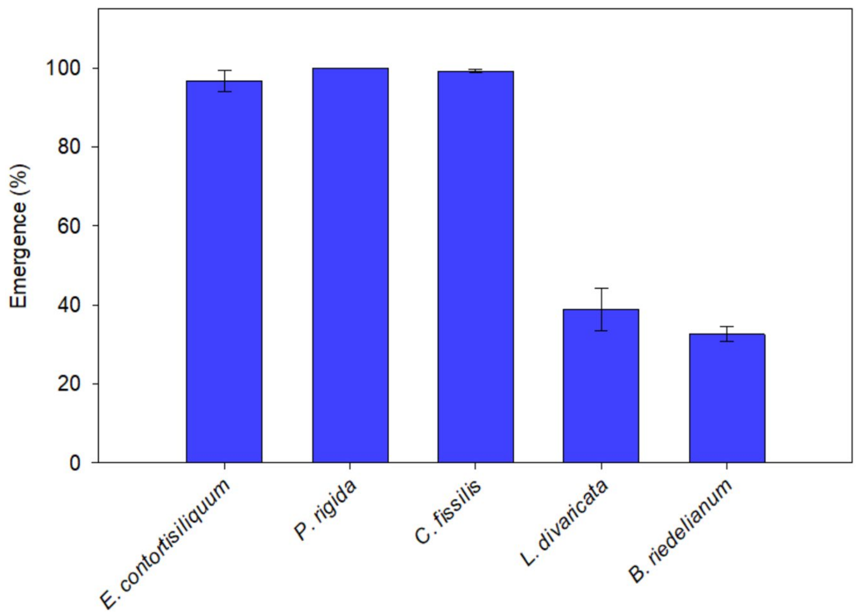

Figure 3. Seedling emergence (mean with standard deviation) of five native forest tree species in greenhouse conditions 60 days after sowing.

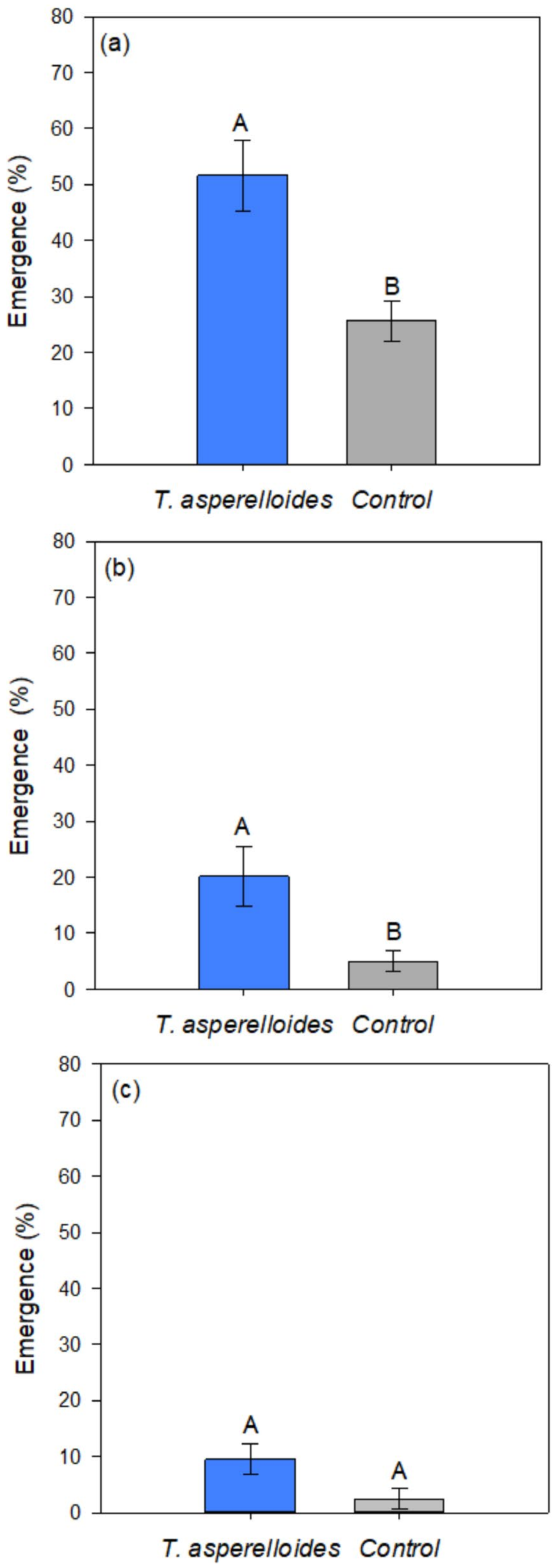

Figure 4. Seedling emergence (mean with standard deviation) of *Enterolobium contortisiliqumm* (a), *Parapiptadenia rigida* (b) and *Cedrela fissilis* (c) 150 days after sowing, with or without seed inoculation. *Means followed by the same letter are not significantly different according to Student's t-test at 5% probability of error.

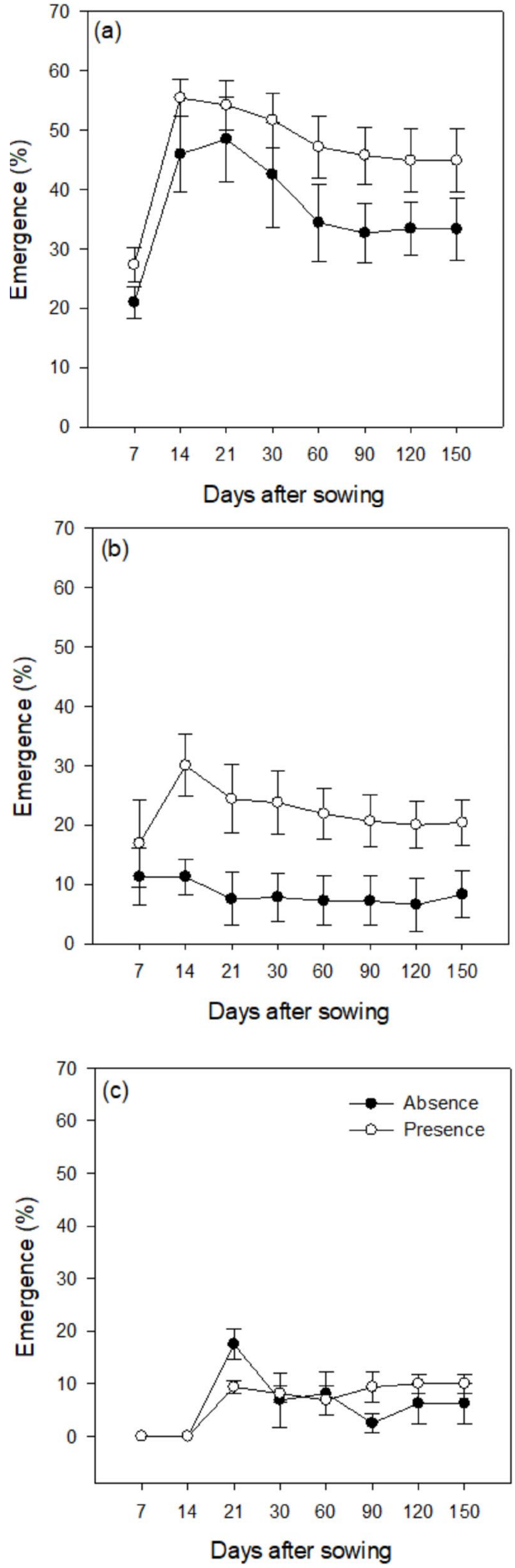

Figure 5. Effect of presence or absence seed inoculation with *Trichoderma asperelloides*on the emergence of seedlings of *Enterolobium contortisiliqumm* (a), *Parapiptadenia rigida* (b) and *Cedrela fissilis* (c) throughout 150 days after sowing. Vertical bars indicate the standard deviation.

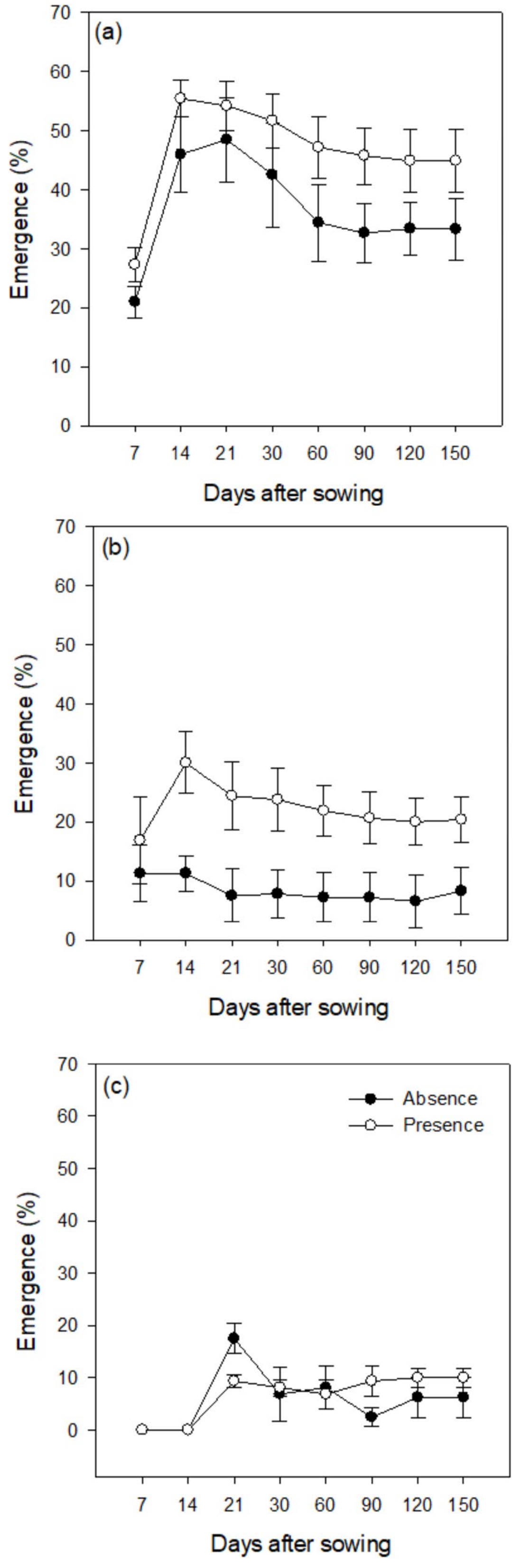

Figure 6. Seedling survival (mean with standard deviation) of five native forest tree species at 360 days after sowing in the field.

E. contortisiliqumm, *P. rigida* and *C. fissilis* were classified as having adequate adaptability (with survival greater than 10.0 %), when used in the direct sowing technique, while *L. divaricata* and *B. riedelianum* were classified as failed species, as they did not present emergence in the field.

Initial plant growth

A significant effect of the inoculation was observed only on the growth in height of *E. contortisiliquum*, being about 42. 3% higher than the control treatment (48.3 cm). It is noteworthy that for *P. rigida* and *C. fissilis*, although statistically equal the inoculation, indicated values higher than the one of the control group (Table 2).

DISCUSSION

The emergence of the seeds of *Enterolobium contortisiliquum*, *Parapiptadenia rigida* and *Cedrela fissilis* in greenhouse conditions demonstrated that the seed batch used showed high viability and physiological quality. Whereas for *Luehea divaricata* and *Balfourodendron riedelianum*, the low emergence rates (38.8 % and 32.1 %, respectively), demonstrated that both are species with restriction defined by the reduced viability of the seeds. These results are within the range described by Carvalho, (2003) who reports that the germinative power of seeds of *L. divaricata* and *B. riedelianum* is quite variable and irregular, ranging from 20.0 % to 85.0 %. Thus, the characteristic of both species, confirmed by the low emergence rate in the nursery, explains the results obtained in the field, where environmental restrictions are more evident.

The mean seedling emergence differed among the five study species, with an overall mean of 11.2 %, being well below the one observed in the greenhouse (73.4 %). These results corroborate with other studies that highlight that a low percentage of emergence and large variations between species in direct sowing is common. Souza and Engel (2018) found that among 31 native tree species tested, only 10 had emergence rates above 10.0 %. Palma and Laurance (2015) in a review of 120 studies carried out with the use of direct sowing in the field in different ecosystems, found that the mean seedling emergence was 18.0 %. Bertacchi et al. (2016) evaluating the establishment of seedlings of nine tree species in the enrichment via direct sowing of a 10-year-old tropical forest, observed an mean emergence of 35.3 %. Analyzing the species for their germination potential in Brasil (2013), we found that only 22.0 % had no germination restriction (dormancy and germination unevenness), that is, the seed technology was known. In the present study, three of the five species are not limited to germination under controlled conditions and application of pre-germination treatments when necessary, however, biotic and abiotic factors can influence the direct sowing.

Table 2: Height and stem diameter of seedlings of tree native forest species due to inoculation with *Trichoderma asperelloides*.

Species	Height (cm)		Stem diameter (mm)	
	T. asperelloides	Control	*T. asperelloides*	Control
C. fissilis	31.25 A	19.00 A	10.95 A	7.22 A
E. contortisiliquum	68.64 A	48.25 B	11.01 A	9.62 A
P. rigida	22.13 A	15.30 A	2.91 A	2.99 A

*Means followed by the same letter on the line are not significantly different according to Student's t-test at 5% probability.

According to Souza and Engel (2018), low seedling emergence is the main barrier to overcome to guarantee the establishment of forest community. Several factors can influence the seed germination process in the field and, consequently, the success of direct sowing (Jesus et al., 2017). According to Oliveira and Barbosa (2014), the factors that influence the germination process can be classified as internal (longevity and viability) and external, related to environmental conditions, such as humidity, temperature, light and oxygen. Thus, the propensity to use certain species using the direct sowing technique can be limited, as observed for *L. divaricata* and *B. riedelianum*.

When analyzing the emergence accumulated over the evaluation period (Figure 5), it is possible to confirm the adverse effects of the climatic conditions in the initial phase of the study. At the beginning of October 2017, we observed a high rainfall in the area, reaching 243 mm (Figure 2), and soaking soil. We verified that this event caused the deterioration of seeds with permeable integument (*P. rigida*, *C. fissilis* and *B. riedelianum*), loss of seedlings of *E. contortisiliquum*, *P. rigida* and *C. fissilis* and burial of seeds of *L. divaricata* and *B. riedelianum*, which have seeds with reduced size or require longer time for germination, respectively. In addition, the situation may have worsened in the following months (spring-summer), when precipitation significantly decreased, raising the temperature, because although there is a forest cover, it was still in formation, which probably led to the dryness of the soil surface.

Other studies have also highlighted the adverse effects of climatic variables on direct sowing. Jesus et al. (2017) found low emergence rates after the occurrence of large volumes of precipitation, due to the flooding of the pits, the deterioration and burial of the seeds, especially for species with small seeds. Soares and Rodrigues (2008) also reported that seeds and seedlings are affected by climatic factors, and that non-adapted species tend to suffer high mortality rates. These results show that climatic seasonality can be an important constraint in the establishment of forest species in direct sowing projects, especially in the first 30 days after implantation. In addition, the selection of species for this purpose should also be carried out, based on species with larger seeds and with known technology of production.

The use of the fungus *T. asperelloides* in the treatment of seeds proved to be a promising strategy to increase the emergence of *E. contortisiliquum* and *P. rigida*. The emergence of seedlings of *E. contortisiliquum* in the field increased from 25.6 % to 51.7 % with the use of *T. asperelloides*, while for *P. rigida* the increase was from 5.0 % to 20.2 % (Figure 4). In addition, *C. fissilis*, *E. contortisiliquum* and *P. rigida* presented the highest heights in plots submitted to the application of *T. asperelloides*.

The benefits caused by the use of fungi of the genus *Trichoderma* are related to the ability of some isolates to act in the control of pathogens associated with seeds and to induce or stimulate a greater synthesis of phytohormones or other substances, such as indoleacetic acid, cytokinin and gibberellin (Chagas et al., 2017; Gravel et al., 2007). These substances help to increase the availability, absorption and efficiency in the use of some nutrients by the plant (Doni et al., 2013), which are used in the production of ATP and NADPH and consequently in the allocation of biomass.

Beneficial results from the application of *Trichoderma* spp. such as significant increases in the percentage and emergence speed index, as well as increased plant growth, have been widely reported in agricultural crops (Guler et al., 2016; Racic et al. 2018; Sankar et al., 2019; Saravanakumar et al., 2013). With regard to the study with forest species, there is no research related to the use of *Trichoderma* as a way to maximize emergence in direct sowing in the field, with some studies being developed in controlled environments. Peccatti et al. (2019) found positive responses from the inoculation with *T. asperelloides* in the production of seedlings of *Maytenus ilicifolia* Mart. ex Reiss in a nursery. Amaral et al. (2017) evaluating seedlings of *Jacaranda micrantha* Cham. in a controlled environment, observed greater growth when they were cultivated on substrate with the presence of *T. asperelloides*.

For the species *C. fissilis* there was no significant effect of the application of *T. asperelloides* on the emergence. This probably occurred because the mechanisms of action of ***Trichoderma*** can vary according to the species, the type of cultivation and the environmental conditions (availability of nutrients, pH or temperature) (Benítez et al., 2004). Similarly, Junges et al. (2016) also found that the emergence and initial development of seedlings of *C. fissilis* were not influenced by the seed treatment with *Trichoderma* spp. The form of inoculation of *Trichoderma* is also a factor worth mentioning, since it is not standardized among the studies (Azevedo et al., 2017), and other forms of application should be tested as inoculation in the substrate, in the planting furrow or in organic materials.

As for the application of hydrogel in the sowing pit, we did not observe any significant effect on the evaluated attributes. Such a situation is possibly associated with the intensity of the precipitations immediately after the implementation of the experiment (Bdmep-Inmet, 2020), which ensured the maintenance of soil moisture around the seeds. Souza et al. (2020) evaluating the effect of the use of hydrogel on the emergence and growth of 15 forest species in the field, also found that the polymer did not influence species performance. Thus, when direct sowing is carried out in places with a low level of degradation and periods prone to high levels of pluviometric precipitation, the use of the hydrogel can be dispensed.

Although the field emergence was low, survival was high for all species that emerged, with values above 87.0 %, despite the damage seen in some seedlings due to low temperatures and the occurrence of frosts in winter. These results suggest that once the first adverse ecological filters were overcome, the development of the species studied was enhanced. Factors such as the isolation of the site, with a protection fence avoiding the presence of herbivorous animals, and ant control, also favored this result. This fact has been verified in other studies evaluating the performance of different species submitted to direct sowing (Sangsupan et al., 2018; Souza and Engel, 2018).

Thus, with regard to the suitability of species for direct sowing, defined according to their mean survival rate at 12 months after sowing, *E. contortisiliqumm*, *P. rigida* and *C. fissilis* were classified as adequate, demonstrating themselves able to be used in direct sowing projects. According to Souza and Engel (2018), use of suitable species in direct sowing projects implies a smaller number of seeds, reducing costs in the technique. In addition, these authors report that species considered adequate generally have medium to large size seeds, corroborating the results obtained in the present study, except for the species *B. riedelianum*, which although having medium-sized diaspores, was classified as a failure.

Based on the results obtained in the present study, considering the practical terms of application of the technique, using five inoculated seeds per pit, we estimate that after a year of implantation, to obtain a minimum density of 100 seedlings (≅ 40.6 cm in height and 8.3 mm in diameter) of the species *E. contortisiliqumm*, *P. rigida* and *C. fissilis* per hectare, it would be necessary to acquire and sow 1,375, 4,132 and 9,843 seeds per hectare, respectively, which corresponds to approximately 458, 138 and 280 grams of seeds.

Although the present study tested only five native tree species, the results indicate the possibility of enriching areas of the Atlantic Forest that are in the process of restoration, through direct sowing, however, at first it should be combined with complementary techniques, such as the planting of seedlings. Although this study is the first to evaluate the use of *T. asperelloides* in direct sowing, the results in the field suggest that this action represents a viable and important sustainable strategy to optimize the emergence and initial growth rates, as well as to overcome the adversities found in the beginning of seedling establishment. Research aiming to expand the knowledge regarding a greater number of species that can be used, physiological characteristics of the seeds, conditions of microsites, different periods of sowing and efficiency of other isolates and *Trichoderma* spp. based products can contribute to the expansion of successful use of direct sowing in subtropical regions.

CONCLUSIONS

Direct sowing proved to be a promising technique for the enrichment of young restoration plantations in subtropical regions with *Enterolobium contortisiliquum*, *Parapiptadenia rigida* and *Cedrela fissilis*. *Balfourodendron riedelianum* and *Luehea divaricata* seeds did not germinate in the field. The use of *Trichoderma asperelloides* represents an efficient strategy to enhance the results of enrichment direct sowing, capable of providing higher emergence rates for *Enterolobium contortisiliquum* and *Parapiptadenia rigida* and increasing the growth in height of *Enterolobium contortisiliquum*. The use of the hydrogel in the enrichment direct sowing, in periods of high pluviometric intensity, did not influence the emergence, survival and morphological attributes of the species studied.

ACKNOWLEDGMENTS

The authors would like to thank Coordination for the Improvement of Higher Education Personnel (CAPES) for granting scholarship to the first author and to the National Council for Scientific and Technological Development (CNPq) for the scholarship of productivity in research provided to the second author.

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