

**DIRECT SEEDING FOR FOREST RESTORATION IN SOUTHERN BRAZIL:
INFLUENCE OF SOIL CONDITIONS AND SUBTROPICAL CLIMATE****SEMEADURA DIRETA PARA RESTAURAÇÃO FLORESTAL NO SUL DO BRASIL:
INFLUÊNCIA DAS CONDIÇÕES DO SOLO E DO CLIMA SUBTROPICAL**

Bruna Balestrin Piaia,
<https://orcid.org/0000-0001-7639-6978>¹
Ana Paula Moreira Rovedder,
<https://orcid.org/0000-0002-2914-5954>²
Idiane Fátima Giacomini,
<https://orcid.org/0000-0002-3612-3841>³
Roselene Marostega Felker,
<https://orcid.org/0000-0003-3944-4381>⁴
Maureen de Moraes Stefanello,
<https://orcid.org/0000-0001-7748-3969>⁵
Rafaela Badinelli Hummel,
<https://orcid.org/0000-0002-3251-6031>⁶
Ricardo Bergamo Schenato,
<https://orcid.org/0000-0003-4268-7691>⁷

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Abstract

In this study, we evaluated the direct seeding of tree Atlantic Forest species for ecological restoration under subtropical climate. We aimed to answer: Are the seedling survival and development influenced by the low temperatures in the winter or soil conditions? Which species have the best development and are most suitable for restoration projects in the subtropical region? The study was carried out in a degraded riparian forest in the center of the Rio Grande do Sul state, southern Brazil. The seeded species were *Schinus terebinthifolia* Raddi, *Psidium cattleianum* Sabine, *Eugenia uniflora* L., *Cupania vernalis* Cambess., *Prunus myrtifolia* L. (Urb.), *Zanthoxylum rhoifolium* Lam. Evaluations were performed monthly for 12 months. Generalized linear models (GLM) were evaluated using emergence, survival and seedling height as response variables, also species and time as explanatory variables with the Gamma distribution. The species that germinated were *P. cattleianum*, *E. uniflora* and *C. vernalis*. *Eugenia uniflora* presented a

¹Doutora em Engenharia Florestal, Programa de Pós-graduação em Engenharia Florestal, Universidade Federal de Santa Maria, brunabpiaia@gmail.com

²Doutora em Ciência do Solo, Departamento de Ciências Florestais, coordenadora do Núcleo de Estudos e Pesquisas em Recuperação de Áreas Degradadas - NEPRADE, Universidade Federal de Santa Maria, anarovedder@gmail.com

³Mestra em Engenharia Florestal, Programa de Pós-graduação em Engenharia Florestal, Universidade Federal de Santa Maria, idiane.giacomini@gmail.com

⁴Doutora em Engenharia Florestal, Instituto de Meio Ambiente de Santa Catarina (IMA), marostegafelker@gmail.com

⁵Doutora em Engenharia Agrícola, Programa de Pós-graduação em Engenharia Agrícola, Universidade Federal de Santa Maria, mmstefanello@gmail.com

⁶Doutora em Engenharia Agrícola, Programa de Pós-graduação em Engenharia Agrícola, Universidade Federal de Santa Maria, rafaela.hummel@gmail.com

⁷Doutor em Ciência do Solo, Departamento de Solos, Universidade Federal de Santa Maria, ribschenato@gmail.com

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higher germination rate (83.33%) and survival (80.00%) at 360 days after sowing. The species did not differ in height growth. We noted differences in height development over time caused by winter conditions. *Cupania vernalis* and *E. uniflora* may be considered as potential species for direct seeding in their natural occurrence areas in southern Brazil. Our study highlights the importance of enhancing ecological aspects of direct seeding.

Keywords: Seedling emergence. Survival. Low temperatures. Soil quality.

Resumo

O objetivo deste estudo foi avaliar a semeadura direta de espécies arbóreas da Floresta Atlântica para restauração ecológica em clima subtropical. Objetivou-se responder: A sobrevivência e o desenvolvimento das mudas são influenciados pelas baixas temperaturas do inverno ou pelas condições do solo? Quais espécies têm melhor desenvolvimento e são mais adequadas para uso em projetos de restauração na região subtropical? O estudo foi realizado em uma mata ciliar degradada no centro do estado do Rio Grande do Sul, sul do Brasil. As espécies semeadas foram *Schinus terebinthifolia* Raddi, *Psidium cattleianum* Sabine, *Eugenia uniflora* L., *Cupania vernalis* Cambess., *Prunus myrtifolia* L. (Urb.), *Zanthoxylum rhoifolium* Lam. As avaliações foram realizadas mensalmente durante 12 meses. Modelos lineares generalizados (GLM) foram avaliados utilizando emergência, sobrevivência e altura de plântulas como variáveis resposta e espécies e tempo como variáveis explicativas a partir da distribuição Gama. As espécies que germinaram foram *P. cattleianum*, *E. uniflora* e *C. vernalis*. *Eugenia uniflora* apresentou maior taxa de germinação (83,33%) e sobrevivência (80,00%) aos 360 dias após a semeadura. As espécies não diferiram quanto ao crescimento em altura. Notou-se diferenças no desenvolvimento em altura ao longo do tempo, causadas pelas condições de inverno. *Cupania vernalis* e *E. uniflora* podem ser consideradas espécies potenciais para semeadura direta em suas áreas de ocorrência natural no sul do Brasil. Esse estudo destaca a importância de melhorar os aspectos ecológicos da semeadura direta.

Palavras-chave: Emergência. Sobrevivência. Baixas temperaturas. Qualidade do solo.

1. INTRODUCTION

The conversion of natural vegetation to anthropogenic uses leads to loss of habitat and connectivity (BOESING et al., 2018), loss of biodiversity, its products and services (ROVEDDER et al., 2016), loss of soil functions and other services such as climate regulation and the hydrological cycle (De GROOT et al., 2002). Several agreements globally, such as Bonn Challenge and the New York declaration, have been signed to guaranteeing the natural heritage and the different services provided by ecosystems, as carbon sequestration and the climate change mitigation. These agreements have placed ecological restoration as one of the most important ways to mitigate the impact of global ecosystem degradation (TEMPERTON et al., 2019). The United Nations (UN) declared 2021-2030 as the 'United Nations Decade for Ecosystem Restoration', in order to intensify and encourage the restoration of ecosystems. Ecological restoration provides solutions to environmental problems reconstructing the structure and functionality of biological communities (MARTIN, 2017). However, it needs to be cost-effective (KIMBALL et al., 2015), tailored to the needs of rural communities and regional cultural characteristics (ARONSON et al., 2011).

Direct seeding for ecological restoration can be an alternative for implantation cost reduction (SOUZA and ENGEL, 2018; RAUPP et al., 2020), in addition to being easily executed



(CECCON et al., 2016), mainly in remote regions. The technique has presented satisfactory results in Brazil, such as in the Cerrado (SILVA et al., 2015, PELLIZZARO et al., 2017) and in the Atlantic Forest biomes (AGUIRRE et al., 2015; COELHO et al., 2016). However, there are still uncertainties in the implementation of direct seeding which may limit its use. The scarcity of viable seed lots, lack of information about the appropriate season for sowing, variability in germination time, impossibility to control environmental characteristics suitable for germination, seeds and seedlings predation and high competitiveness in early stages of development, long dry season effects on the survival and success rate are the main limits (BRANCALION et al., 2016; CECCON, et al., 2016). In addition, most of the direct seeding projects were carried out in a tropical climate (PALMA and LAURANCE, 2015; CECCON, et al., 2016), so, we have a lack of information about this technique in the subtropical climate.

Moreover, there is a general lack of scientific information regarding the specificities of restoration strategies in the southern region of Brazil, which has a subtropical climate (ROVEDDER et al., 2014). The history of suppression and conversion of natural areas, as well as studies on the natural regeneration potential (MMA, 2017) show the urgency of policies and programs that are based on ecological restoration principles. Knowledge of the dynamics of restoring subtropical ecosystems can fill the gap of scientific information for ecological conservation, which is an important premise for sustainable development.

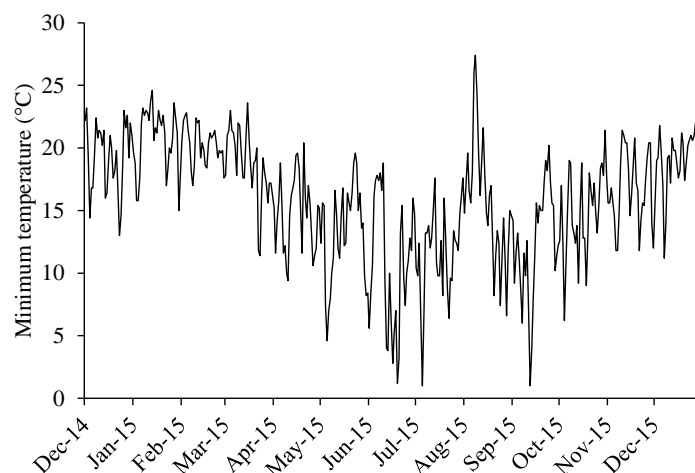
Therefore, it is important to test ecological restoration techniques for the reality of subtropical Brazil, where the responses will be mainly conditioned by temperature variation. In this study, we evaluated the direct seeding of Atlantic Forest species for ecological restoration under subtropical climate. We aimed to answer: Are the survival and development of seedlings influenced by the low temperatures in the winter or soil conditions? Which species have the best development and are most suitable for restoration projects in the subtropical region?

2. MATERIAL AND METHODS

2.1 Study site

The study was carried out in a riparian ecosystem in the Atlantic Forest, in the central region of Rio Grande do Sul state, southern Brazil (29°35'13"S and 53°46'58"W). The climate of the region is Cfa type, subtropical humid, according to the Köppen classification, with rains well distributed throughout the year and hot summers. The average temperature of the coldest month is 12.2 °C and the hottest month is 22.9 °C; the average monthly rainfall ranges from 136 mm to 191 mm and the average altitude is 400 m (ALVARES et al., 2013). We obtained minimum daily temperatures for the region during the study period (December 2014 to December 2015) from the Brazilian meteorological database of teaching and research of the Instituto Nacional de Meteorologia - INMET (Figure 1).



Figure 1. Minimum daily temperatures during the study period. Data source: INMET.

The main regional phytogeographic type is Seasonal Forest in contact with the Mixed Ombrophilous Forest (MARCHIORI, 2002). Degradation of the area occurred by suppression of the original vegetation (riparian forest) and substitution by subsistence agricultural cultivation. The study site was 0.5 ha and was abandoned about 12 months before the experiment. There was scarce vegetation cover at the time of direct seeding, consisting of spontaneous herbaceous species. The relief of the study area is flat and the predominant soil classes in the landscape are Cambisol and Leptosol in association with Regosol (STRECK et al., 2008).

2.2 Tree species, site preparation and experimental design

We selected six regional tree native forest species for direct seeding based on a floristic survey of a reference forest (Table 1). This reference forest was located within the same phytophysiognomy and watershed (PIAIA et al., 2015). The seeds were collected in the study region and were stored in a cold chamber. None of the selected species required performing dormancy breakdown for their emergence.

Table 1. Ecological group and seed size of the sown regional native tree species.

Scientific name	Family	Ecological Group*	Seed size**
<i>Schinus terebinthifolia</i> Raddi	Anacardiaceae	P	S
<i>Psidium cattleianum</i> Sabine	Myrtaceae	CL	S
<i>Eugenia uniflora</i> L.	Myrtaceae	CL	L
<i>Cupania vernalis</i> Cambess.	Sapindaceae	CS	L
<i>Prunus myrtifolia</i> L. (Urb.)	Rosaceae	CL	M
<i>Zanthoxylum rhoifolium</i> Lam.	Rutaceae	P	S

In which: *P = Pioneer; CL = climax demanding light; CS = climax shade tolerant [Classification proposed by Swaine & Whitmore (1988) and adapted by Oliveira Filho et al. (1994)]; **Seed size based on seed weight: S=Small (< 0.069 g), M=Medium (0.07–0.39 g), L=Large (more than 0.40 g).

A subsoiler was first used to unpack the topsoil to prepare the sowing area. Next, we removed the weeds within a 60 cm radius of each spot by manually weeding. We performed

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sowing in December 2014. The experiment was conducted in a completely randomized design, in which the experimental units were the spots arranged in rows and the treatments were the species, with 20 replicates. The distance between the lines was 2 m and between the spots on the line was 1 m. The lines were within two meters of the stream. The species were randomly distributed, all seeds were inserted at 2 cm of depth and covered with soil, with three seeds sown at each spot. There was no soil fertilization. The area was fenced to prevent free access to animals. Nests or attacks by leaf-cutting ants were not recorded. We monthly removed and cleaned spontaneous herb species.

2.3 Soil conditions

Chemical and physical analyses were performed to characterize the soil conditions. For chemical analysis, four samples were extracted from five subsamples each, at depths of 0 to 0.1 m and 0.1 to 0.2 m. The content of macronutrients and aluminum, pH, saturation by aluminum (m%), saturation by bases (V%), effective and potential cation exchange capacity and organic matter were analyzed.

The physical properties of field capacity, permanent wilt point, hydraulic conductivity (K), bulk density, total porosity, macroporosity and microporosity were obtained from undisturbed samples. Field capacity estimated in a sand column at 10 kPa; wilting point estimated in psychrometer (WP4) at 1500 kPa; hydraulic conductivity measured in constant load permeate. Soil density was obtained by a volumetric ring; particle density obtained by volumetric flask; total porosity is a relationship between soil density and particle density; microporosity obtained by a balanced water content with a matrix potential of -60 cm; macroporosity is the difference between total porosity and microporosity.

The soil resistance to penetration was evaluated using an impact penetrometer at depths 0 to 10 and 10 to 20 cm. The value obtained represents the average of five measurement points. The granulometric analysis was performed using the pipette method (EMBRAPA, 1997). Chemical analyses were carried out at the Soil Analysis Laboratory (SAL) and physical analyses were carried out in the Soil Physics Laboratory, both at the Universidade Federal de Santa Maria (UFSM).

2.4 Data collection and analyses

We monitored the experiment for 12 months post-seeding. Germination was evaluated by counting seed emergence by spot at monthly intervals. The percentage of germination and survival for seeded species were determined monthly. We measured the seedling height after reaching a height greater than or equal to 3 cm to evaluate the species development.

Generalized linear models (GLM) were evaluated using emergence, survival and seedling height as response variables and species and time as explanatory variables with the Gamma distribution. The GLM models were built using the “glm” function in the R environment version 3.6.1 (R Core Team, 2019).

3. RESULTS

3.1 Seed germination and development

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We sowed 360 seeds, considering the six species analyzed and the number of seeds per spot, only 62 germinated, being 83.33% of the *E. uniflora* seeds, 18.33% of the *C. vernalis* seeds (Figure 2), and 0.05% of the *P. cattleyanum* seeds. The other species did not germinate. After 12 months of evaluation, a total of 47 seedlings survived, meaning 13.05% of the seeds were successful in one year.

Figure 2. *Eugenia uniflora* (a) and *Cupania vernalis* (b) seedling development in a degraded riparian forest area in southern Brazil.



Psidium cattleyanum germinated at 30 days with 100% mortality at 90 days. Therefore, we only made comparisons for *E. uniflora* and *C. vernalis*, which germinated at 90 days after sowing. *Cupania vernalis* showed an increasing number of seeds germinated up to 180 days, and *E. uniflora* up to 210 days; we later observed seed emergence stabilization (Figure 3a). The species emergence was statistically different ($t=-6.420$; $p<0.0001$). The emergence over time was also statistically different for each species ($t=-2.323$; $p=0.0207$), but the interaction of species and time was not significant ($t=2.003$; $p=0.0459$).

Eugenia uniflora presented more seedling survival ($t= -3.804$; $p= 0.000167$) (Figure 3b). We did not observe statistically difference of survival over time ($t= 1.476$; $p= 0.140724$) and in the interaction of species and time ($t=-1.534$; $p= 0.125852$).

The species did not differ in height ($t=-1.477$; $p=0.1406$), but *C. vernalis* presented a decrease at 180-210 days. *Eugenia uniflora* presented growth stabilization in the same period and high growth in the following months ($t=1.790$, $p=0.0743$) (Figure 4). The species presented different height development over time ($t=-1.906$; $p=0.0575$).

Figure 3. Cumulative seed germination (a) and seedling survival (b) at 360 days after sowing in a degraded riparian forest area in southern Brazil.

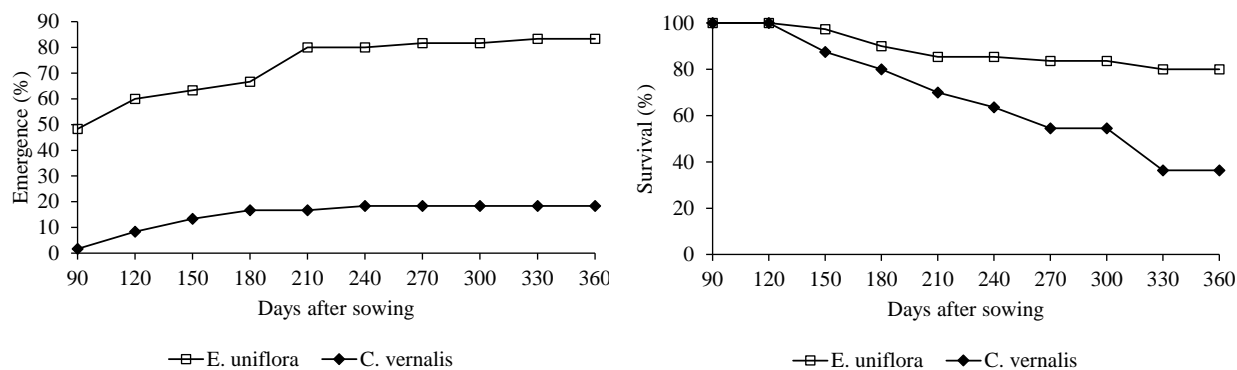
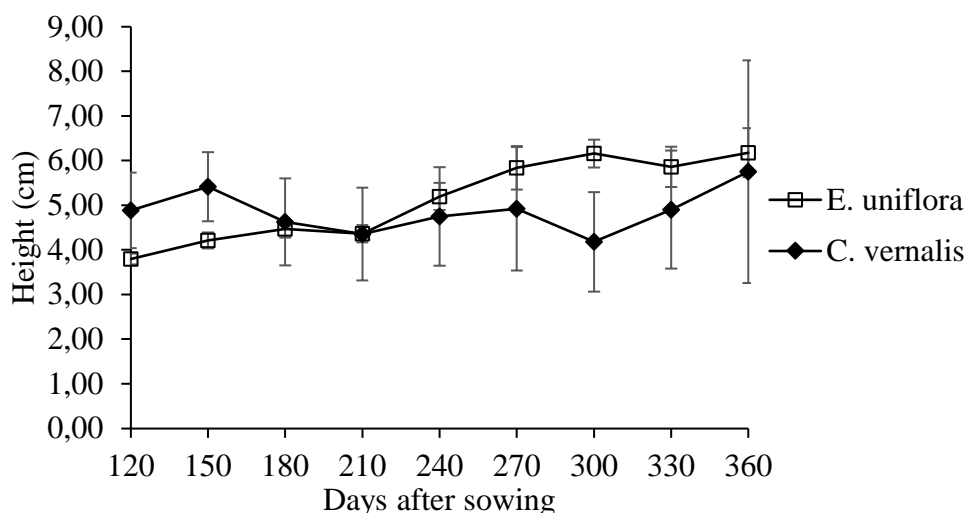


Figure 4. Average height (cm) of seedlings at 360 days after sowing in a degraded riparian forest area in southern Brazil.



3.2 Soil conditions

The study site presents a high concentration of macronutrients resulting from the fertilization of the last cultivation period; however, it presents low effective CEC, potential CEC, base saturation and organic matter values (Table 2).

Table 2. Chemical and physical soil variables at the depths of 0-0.1 and 0.1-0.2 m in a direct seeding site, southern Brazil.

Chemical variables	Depth (m)	pH	P	K	Al	Ca	Mg	CEC ef	CEC pH7	V	m	OM	Clay
			mg/L			cmolc/L							
	0 – 0.1	5.4	12.0	238.0	0.2	6.1	2.4	9.4	13.5	67.7	3.0	2.9	30.0
	0.1 – 0.2	4.8	6.3	171.0	1.8	4.1	1.5	7.8	15.1	42.5	24.5	2.4	33.8

Physical variables	Depth (m)	FC	PWP	K	D	MaP	MiP	TP	RP	Particle size (%)			
		(cm ³ cm ⁻³)	(cm ³ cm ⁻³)	(mm h ⁻¹)	(g cm ⁻³)	(cm ³ cm ⁻³)	(cm ³ cm ⁻³)	(cm ³ cm ⁻³)	(Mpa)	Coarse sand	Thin sand	Silt	Clay
	0 – 0.1	0.4	0.2	252.7	1.2	0.1	0.4	0.5	1.8	12.4	16.9	43.1	27.7
	0.1 – 0.2	0.4	0.2	452.1	1.3	0.1	0.4	0.5	2.3	14.3	17.7	40.6	27.5

*In which: m: aluminum saturation (%); V: base saturation (%); CEC. ef.: Effective Cation Exchange Capacity; CEC pH 7: Potential Cation Exchange Capacity; OM: Organic Matter; FC: Field capacity (cm³/cm³); PWP: Permanent Wilting Point (cm³/cm³); k: hydraulic conductivity (mm/h); D: Bulk density (g/ cm³); MaP: macroporosity (cm³/cm³); MiP: microporosity (cm³/cm³); TP: Total Porosity (cm³/cm³); RP: Soil Resistance to penetration (Mpa).

4. DISCUSSION

Low values of species establishment have also been observed in other recent studies using direct seeding of forest species for ecological restoration (PALMA and LAURANCE, 2015; CECCON et al. 2016; MELI et al., 2018). One study that evaluated the germination success of 86 forest species by direct seeding from 30 papers developed in 13 countries, in which 32.5% were carried out in Brazil, verified that most species (72%) presented a low probability of success (CECCON et al., 2016). The authors recommended the use of direct seeding together with other restoration techniques, and the selection of species in the direct seeding projects should be done with caution for a favorable cost/benefit ratio (CECCON et al., 2016). This means that a large number of seeds would guarantee a good recovering, however this would bring unnecessary additional costs. In terms of successional stage, pioneer species can show better germination and development rates and provide good recover in the early stages. For this, a smaller number of species can be used aiming recovery sowing and then an additional sowing can be carried out aiming the diversity enrichment (BRANCALION et al., 2015).

The lack of answers for *S. terebinthifolia*, *P. myrtifolia* and *Z. rhoifolium* may be related to the viability and vigor of the seed lot. They could be at the threshold of low physiological quality. This same fact may have been the cause of the low germination of *P. cattleyanum*. It is a consensus in the Brazilian scientific community that a lack of forest tree seeds with good levels of physiological quality in sufficient quantities is one of the market gaps linked to the current demands for ecosystem restoration projects in the country (BRANCALION et al., 2015). It is worth mentioning that Brazil is a signatory to important global agreements such as the Bonn Challenge and the Paris Agreement, and it is committed to restoring 12 million hectares by 2030 (BRAZIL INDC, 2015). Despite the growing market demand for seeds of native forest species, many of these species still have limitations due to a lack of knowledge regarding morphological and ecophysiological characteristics (GUOLLO et al., 2017).

The germination and survival of the seeded species may be associated with the size of the seeds. Their accumulated reserves influence the emergence and development of the seedlings (FERREIRA et al., 2009). This condition could increase the chances of survival in water stress, unfavorable light intensity conditions and soil nutrient shortage (SILVA and CARVALHO, 2008). In this sense, we can relate the results obtained for *E. uniflora* and *C. vernalis* to the larger size of seeds when compared to the other seeded species. This functional trait can be used to guide species selection in restoration projects (MELI et al., 2018). Seed size has been one of the main



characteristics associated to the success of germination and establishment of seedlings in direct seeding projects (TUNJAI and ELLIOTT 2012; SOUZA and ENGEL, 2018).

Another factor that may have conditioned the low germination results may be the depletion of soil quality. The chemical-physical analyses showed high K and P content; a residual effect of the last management period (Table 2). On the other hand, the effective CEC, potential CEC, base saturation and organic matter values show the exhaustion of soil quality (Table 2). This influence of soil quality was verified by the higher germination percentage in more fertile soils (MELI et al., 2018). Ceccon et al. (2016) suggest increasing seeding density in areas with lower soil quality.

The monitoring showed the harmful effect of winter temperatures on height growth, especially for *C. vernalis*, which decreased from 180 days after sowing, corresponding to the months of May-June. This is the beginning period of the cold season in the region, with frost occurring until September (WREGE et al., 2018). The average monthly minimum temperature in the year of the experiment was 13.0 and 10.7°C for May and June, respectively (Figure 1). The occurrence of frost may have caused damage to the apical meristem, and later the leaves falling obstructed its development in height. Some *C. vernalis* seedlings showed decreasing height, which explains the high variability, and could mainly be verified at 300 days after sowing (Figure 4). This period corresponds to October and this result may also be associated with the climate of the region. In September, there was a minimum temperature around 0°C with probable occurrence of frost (Figure 1).

The height growth and greater seed germination of *Eugenia uniflora* may be associated with its ecological group. Because it is a pioneer species, it presents rapid initial growth and good establishment in the field, in addition to being able to withstand the cold well and does not present restrictions on the type of soil (CORADIN et al., 2011).

Although the present research presented scarce satisfactory results, *Eugenia uniflora* and *Cupania vernalis* presented potential for direct seeding in subtropical Brazil in a natural occurrence area. In addition, one of the main reasons for the use of direct seeding as a restoration technique is the low cost of implantation (RAUPP et al., 2020). However, the high levels of uncertainty in using this technique, as well as the limited knowledge about forest seeds, sowing techniques and influences of environmental factors in the establishment of seedlings, may limit their application (BRANCALION et al. 2016). In this sense, it becomes fundamental to develop new research that seeks to investigate the causes that lead to germination, survival and initial development of species to give adequate support to ecological restoration projects (FERREIRA et al., 2007; MELI et al., 2018).

5. CONCLUSION

Cupania vernalis and *Eugenia uniflora* may be considered as potential species for direct seeding to ecological restoration in their natural occurrence areas in southern Brazil. Soil conditions can limit seed emergence and low winter temperatures have influenced the seedling development. The lack of results for the remaining species suggests that other studies should test seed viability and more species under a variety range of conditions. Our study highlights the importance of enhancing the ecological aspects of direct seeding.

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