

EFFECT OF FROST ON GERMPLASM USED IN RESTORATION OF DEGRADED AREA IN THE SERRA DO ITAJAÍ NATIONAL PARK

Carlos Meneghetti^{1*}, Marcelo Diniz Vitorino²

¹ Regional University Foundation of Blumenau, Specialization Course in Recovery of Degraded Areas, Graduate Program in Forestry, Blumenau, Santa Catarina, Brazil – carlosmeneghetti@terra.com.br*

² Regional University Foundation of Blumenau, Department of Forestry, Graduate Program in Forestry, Blumenau, Santa Catarina, Brazil – dinizvitorino@gmail.com

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Abstract

Frosts that occur in the Southern Brazil limit the development and survival of seedlings used for restoration projects and may cause significant losses. The objectives of this study were: evaluating the effect of frosts on seedlings produced by seeds collected around a degraded area inside the Serra do Itajaí National Park (PNSI) and used to restore this area; and validating the use of polyester printed labels for marking seedlings. Planting was carried out in adapted Anderson Groups, with 25 seedlings per module. Survival rates for frost were evaluated in 750 seedlings, identified at the species level, classified according to their ecological group, and used in three areas designated as A, B and C. 80% of the seedlings were planted in sloping area and 20%, in lowland area. For the identification of the seedlings, labels printed by a laser printer were used on matte polyester film and affixed with plastic polyamide clamps. This method proved to be practical, inexpensive and resistant to the climate action (heat, humidity and precipitation). Besides, it does not suffer attacks from the local fauna. The overall survival rate was 55.87%, whereas survival rates by ecological groups were 76.67% for shadow tolerant climax, 53.75% for light demanding climax, and 55.63% for the pioneer. The highest survival rates were the ones from seedlings located on the slopes. It was possible to verify that the local germplasm is well adapted to the frosts that occur in the region.

Keywords: Environmental recovery, adapted Anderson Groups, seedlings tagging, native seedlings, Conservation Units.

Resumo

Efeito da geada sobre germoplasma utilizado em restauração de área degradada no Parque Nacional da Serra do Itajaí. As geadas que ocorrem no Sul do Brasil limitam o desenvolvimento e a sobrevivência de mudas utilizadas em projetos de restauração, podendo causar perdas significativas. Este estudo teve como objetivos: avaliar o efeito provocado pelas geadas em mudas produzidas a partir de sementes coletadas no entorno de uma área degradada, dentro do Parque Nacional da Serra do Itajaí (PNSI), e utilizadas para a restauração desta área; e validar a utilização de etiquetas impressas em poliéster para marcação de mudas. O plantio foi realizado em Grupos de Anderson adaptados, com 25 mudas por módulo. Foram avaliadas as taxas de sobrevivência em relação ao efeito da geada em 750 mudas, identificadas ao nível de espécie, classificadas quanto ao seu grupo ecológico, e utilizadas em três áreas designadas como A, B e C, com 80% das mudas plantadas em área de encosta e 20%, em área de planície. Para a identificação das mudas, foram utilizadas etiquetas impressas por impressora laser em filme de poliéster matte e afixadas com abraçadeiras plásticas de poliamida. Esse método demonstrou ser prático, de baixo custo e resistente à ação do clima (calor, umidade e precipitação), além de não sofrer ataques da fauna local. A taxa de sobrevivência geral foi de 55,87%, enquanto que as taxas de sobrevivência por grupos ecológicos foram de 76,67% para as climáticas de sombra, 53,75% para as climáticas de luz e 55,63% para as pioneiras, sendo as maiores taxas de sobrevivência as das mudas localizadas nas encostas. Foi possível constatar que os materiais genéticos locais estão bem adaptados às geadas que ocorrem na região.

Palavras-chave: Recuperação ambiental, Grupos de Anderson adaptados, etiquetagem de mudas, mudas nativas, Unidades de Conservação.

INTRODUCTION

The selection process of species for seedling production to be used in the restoration of degraded areas should be based on several ecological criteria, such as adaptability to climatic conditions, genetic diversity, fauna attractiveness, rapid growth for soil cover, potential production of organic matter to be incorporated into the soil, among others (REIS *et al.*, 2010; IBAMA, 2013).

An ecological criterion to be observed is the adaptation and resistance of the seedlings to the temperature conditions of the site to be restored, especially in cases where there is a very pronounced thermal amplitude, with very close to zero or even negative temperatures at the sites in which winters are very strict. Therefore, the choice of native species is fundamental, as these species are already adapted to the local ecological conditions and can

therefore avoid problems of climatic adaptation, such as those caused by the occurrence of frost (MARTINS, 2013). Because they are subject to the same temperature conditions as the matrices from which the seeds were collected, it is expected that the seedlings produced from this germplasm present features close to adaptation and resistance to extreme temperature variations.

Considering the scarcity of information and studies on the ecological aspects in the production of seedlings for restoration projects and their relation with the environmental variables, especially in relation to the occurrence of frosts, the objective of this study was: a) to evaluate the survival of the seedlings produced from seeds collected around the area to be restored; b) to verify the influence of the frost on the seedlings sampled; c) to evaluate the influence of frost on the ecological groups planted; and d) to validate a new proposal of low cost and practical methodology to mark and identify seedlings.

MATERIAL AND METHODS

The experiment was conducted in three areas of the National Conservation Unit of the Serra do Itajaí National Park (PNSI), considered the largest continuous area of Atlantic Forest in the state of Santa Catarina, Brazil, and involving nine municipalities: Apiúna, Ascurra, Blumenau, Botuverá, Gaspar, Guabiruba, Indaial, Presidente Nereu and Vidal Ramos (ICMBIO, 2017).

The three areas planted to the restoration project were randomly selected, prior to winter, and are located in the region known as Faxinal do Bepe, in the municipality of Indaial (state of Santa Catarina, Brazil). The central geographic coordinates of each area are: 27° 06' 44" South Latitude and 49° 12' 13" West Longitude for Area A; 27° 06' 40" South Latitude and 49° 11' 48" West Longitude for area B; and 27° 06' 23" South Latitude and 49° 11' 54" West Longitude for area C; with average altitude of 650 m for the three areas. The distances in a straight line between the areas are 650 m between A and B, 800 m between A and C, and 500 m between B and C.

The degraded areas under restoration of this study are in a high degree of anthropization, and this situation occurred mainly due to the type of land use. There is a presence of agriculture in the lowland and pasture areas on hillsides for about 60 years.

The climate of the region, based on the classification of Köppen, is of type Cfa, subtropical humid of hot summer, with more than 40 mm of rain in the driest month, with precipitation between 1,600 and 1,900 mm/year, being the average temperature of the air from the coldest three months is between -3 °C and 18 °C (ALVARES *et al.*, 2013). The occurrence of water deficit and drought periods are not factors that cause great concern, since the climate is humid, with occurrence of significant precipitation in all months of the year and no definite dry season.

The geological formations of Santa Catarina result from a long process of transformations that began about 550 million years ago. The PNSI region consists of lithologies of the Santa Catarina Basin (Escudo Catarinense), which includes older magmatic and metamorphic rocks, sedimentary and volcanic rocks from the Paraná Sedimentary Basin and more recent sediments. In this region, a very varied sequence of rocks occurs and, conditioned by its rugged morphology, has turned it into one of the most fragile and unstable areas of the territory of Santa Catarina. (MMA, 2009). The predominant soils of the region are classified in the group of Cambisols and Gleysols, with inclusions of Argisols and Litholic Neosols (SANTOS *et al.*, 2013).

The vegetation is part of the Atlantic Forest Biome and presents, as the main forest system, the Ombrophilous Dense Forest, with submontana and montana formations, characterized by large arboreal individuals with woody lianas and epiphytes (IBGE, 2012).

The seeds from the selected species for the production of the restoration project seedlings, in which this study was conducted, were collected from matrices located around the degraded area. Its geographic distribution was observed to avoid material originating from related individuals, according to procedures recommended by Shimizu *et al.* (1982), maintaining a minimum distance of 100 meters between matrices of the same population.

The seedlings were classified according to the ecological group to which the species belonged, as described in the study of Oliveira-Filho (1994). In this way, the species were initially produced in a greenhouse from germination to a minimum height of 50 centimeters. They were then taken to flowerbeds with permanent cover and kept at 50% luminosity for at least one month. Afterwards, they were kept in beds in full sun and with a decrease in irrigation for their rustification. All seedlings were produced in black plastic bags, 12 cm in diameter and 26 cm high, with commercial substrates based on Pinus bark, ashes, vermiculite, sawdust and biostabilized, and fertilized with 8 grams of Osmocote® each.

The distribution of the seedlings at the time of planting was done according to the Anderson system (ANDERSON, 1953), in modules that were adapted to the circular format with 25 plants to avoid planting in a straight line, since it is a Conservation Unit of: one shadow tolerant climax (CS), eight light demanding climax (CL) and sixteen pioneers (P) (Figure 1). In addition, the conformation of the module aimed to foment ecological

groups, with the pioneers growing faster, shading the light demanding climax, which, in turn, shade the shadow tolerant climax.

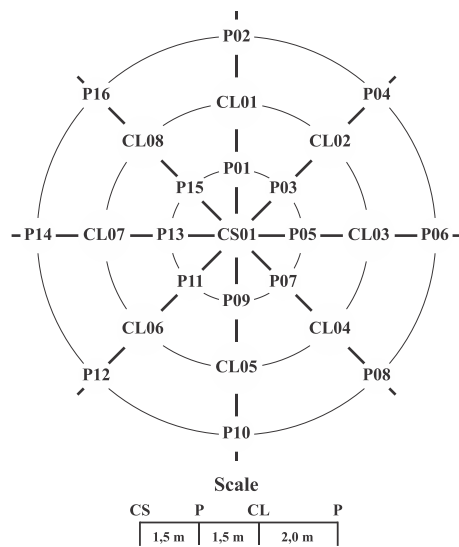


Figure 1. Anderson Group model adapted and used for the planting of the modules.

Figura 1. Modelo de Grupo de Anderson adaptado e utilizado para o plantio dos módulos.

The experimental field part of this study was divided into two distinct stages. The first step consisted in the individual marking of each of the 750 live seedlings by means of printed labels, personalized with an individual reference code.

The labels 20 mm wide by 95 mm long were printed by a laser printer on matte polyester film, 100 micron thick, punched and affixed to each seedling by means of a 200 mm polyamide plastic clamp allowing clearance in the seedling stem (Figure 2).



Figure 2. Polyester label and polyamide clamp (left) and label affixed to the seedling (right).

Figura 2. Etiqueta de poliéster e abraçadeira de poliamida (esquerda) e etiqueta fixada na muda com abraçadeira (direita).

The second stage consisted in the monitoring to verify the survival of the seedlings after the occurrence of frosts in the winter period (Table 1). Climatic data, especially minimum temperatures, were obtained from two meteorological stations of the National Institute of Meteorology (INMET), installed at latitudes and altitudes equivalent to the area of the experiment and located a few kilometers from it. The stations used as reference to obtain the climatic data were: INMET station Ituporanga-A863, code WMO 86957, located at 27 ° 25' 6.276" South and 49 ° 38' 48,746" West, altitude of 480 m; and INMET Station Rio do Campo-A861, code WMO 86944, located at 26 ° 56' 15.094" South and 50 ° 8' 43.602" West, altitude of 592 m (Figure 3).

Weekly monitoring was performed on all seedlings after the occurrence of frosts to evaluate whether they were affected or not, and signs of survival were sought through leaf regrowth or presence of live tips.

This study was developed from the data obtained from 30 plant modules distributed in three areas (10 modules per area), all randomly selected, totaling 750 seedlings. The modules were named from M01 to M10 in area A, M11 to M20 in area B, and M21 to M30 in area C. At the time of marking of the seedlings to the field, there was no identification of the species, being chosen only the modules in which all the seedlings were alive and healthy.

The individual marking and labeling of seedlings occurred on 03/17/2016 for the seedlings of area A; on 03/31/2016 and 04/28/2016 for the seedlings of area B; and on 04/28/2016 and 05/12/2016 for the seedlings of area C. Monitoring for survival of frosts and identification of species occurred weekly for the three areas and was finalized on 12/05/2016 for the seedlings of area A; 12/12/2016 for the seedlings of area B; and 12/12/2016, 12/19/2016 and 01/04/2017 for the seedlings of area C.

The distribution of the seedlings in the field was: 80% in a sloping area with a gentle slope, and 20% in a flat surface area, called plain. The qualitative and quantitative results of the experimental part of this research were analyzed statistically and provided subsidies for the discussion stage, which was performed based on field observation data and specific literature.

RESULTS

The arrangement of the seedlings in the adapted modules of the Anderson system (ANDERSON, 1953) provided a coverage area of 78.5 m² per module (Figure 1).

In the monitoring performed, it was possible to observe that the tags and clamps were intact, resisting the action of weather, and were not damaged or attacked by the local fauna. In addition, it was observed, through regrowth of the seedlings, that frost affected to a greater or lesser extent 100% of the seedlings sampled in this study, causing their defoliation, as observed visually in all seedlings.

During the period from 04/15/2016 to 07/31/2016, seven episodes of frost occurred in the areas where the seedlings were planted, according to *in situ* observations in the experiment area. In Figure 3, the values of the temperatures and the dew point equaled to or were under 5 degrees during the period, acquired in the two meteorological stations of INMET, characterizing the occurrence of frosts are presented.

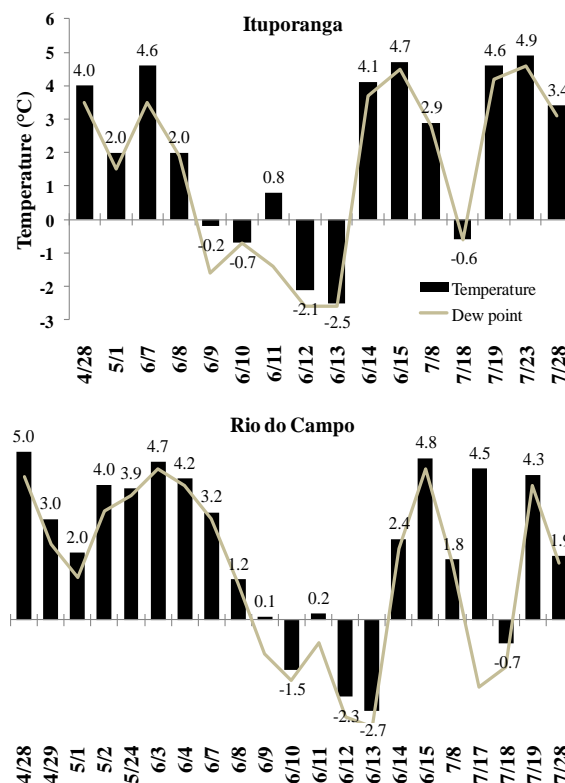


Figure 3. Minimum temperatures of less than or equal to 5 °C and dew point in the period from 04/15/2016 to 07/31/2016 in the meteorological stations of INMET Ituporanga-A863 and Rio do Campo-A861.

Figura 3. Temperaturas mínimas menores ou iguais a 5 °C e ponto de orvalho no período de 15/04/2016 a 31/07/2016 nas estações meteorológicas do INMET Ituporanga-A863 e Rio do Campo-A861.

The overall mortality rate of the seedlings was 44.13%, with 331 dead seedlings, and the overall survival rate of 55.87%, with 419 live seedlings (Table 1 and Figure 4, on the left). Thirty-two species belonging to 17 families were used, distributed among the three ecological groups (Table 1).

Among the 419 seedlings that survived, 30 species were found (Table 1), of which five (16.67%) belonged to the “CS” group, 18 (60.00%) to “CL”, and seven (23.33%) to “P”. Among the 331 seedlings that died, the pioneers represent a total of 213 seedlings (64.35%), of which 97.18% could not be identified.

The survival rates and mortality by ecological group, related to the frost effect, are represented in Figure 4 on the right. “CS” was the ecological group with the highest proportional percentage of survival, with 76.67% of the live seedlings, while “CL” and “P” had a higher mortality, 46.25% and 44.38% respectively.

Table 1. Species by families, ecological groups and number of individuals per species.

Tabela 1. Espécies por famílias, grupos ecológicos e quantidade de indivíduos por espécie.

Family	Species	Ge	Dead	Alive	Total sp.
Annonaceae	<i>Annona sylvatica</i> A. St.-Hil.	CL	-	1	1
Arecaceae	<i>Syagrus romanzoffiana</i> (Cham.) Glassman	CL	-	2	2
	NI	P	84	-	84
Asteraceae	<i>Piptocarpha axillaris</i> (Less.) Baker	P	-	47	47
	<i>Vernonanthura discolor</i> (Spreng.) H.Rob.	P	-	80	80
Euphorbiaceae	<i>Sapium glandulosum</i> (L.) Morong	CL	-	6	6
	<i>Dalbergia brasiliensis</i> Vogel	CL	-	2	2
	<i>Dalbergia frutescens</i> (Vell.) Britton	CL	-	3	3
	<i>Inga marginata</i> Willd.	CL	-	13	13
Fabaceae	<i>Inga sessilis</i> (Vell.) Mart.	CL	2	19	21
	<i>Inga</i> sp. Mill.	CL	32	5	37
	<i>Inga vera</i> Willd.	CL	-	5	5
	<i>Mimosa scabrella</i> Benth.	P	1	5	6
	<i>Muelleria campestris</i> (Mart. ex Benth.) M.J. Silva & A.M.G.	CL	-	6	6
	<i>Senna multijuga</i> (Rich.) H.S.Irwin & Barneby	CL	-	2	2
Lamiaceae	<i>Aegiphila integrifolia</i> (Jacq.) Moldenke	P	-	9	9
	<i>Vitex megapotamica</i> (Spreng.) Moldenke	CL	-	36	36
Lauraceae	<i>Cryptocarya aschersoniana</i> Mez	CL	-	1	1
Lythraceae	<i>Lafoensia vandelliana</i> Cham. & Schtdl.	CL	-	4	4
Melastomataceae	<i>Miconia cabucu</i> Hoehne	CL	22	-	22
Meliaceae	<i>Cedrela fissilis</i> Vell.	CL	2	6	8
	<i>Campomanesia guaviroba</i> (DC.) Kiaersk	CS	-	15	15
	<i>Campomanesia reitziana</i> D.Legrand	CS	-	4	4
Myrtaceae	<i>Eugenia involucrata</i> DC.	CS	-	1	1
	<i>Myrcia brasiliensis</i> Kiaersk.	CS	-	5	5
	<i>Myrcia</i> sp. DC.	CS	-	1	1
	NI	CS	1	-	1
NI	NI	P	123	-	123
	NI	CL	46	-	46
Phytolaccaceae	NI	CS	6	-	6
	<i>Phytolacca dioica</i> L.	P	5	16	21
Primulaceae	<i>Myrsine coriácea</i> (Sw.) R.Br. ex Roem. & Schult.	P	-	1	1
Rosaceae	<i>Prunus myrtifolia</i> (L.) Urb.	CL	6	11	17
Salicaceae	<i>Xylosma pseudosalzmanii</i> Sleumer	CL	-	1	1
Sapindaceae	<i>Cupania vernalis</i> Cambess.	CL	1	-	1
Solanaceae	<i>Solanum mauritianum</i> Scop.	P	-	109	109
	<i>Solanum sanctae-catharinae</i> Dunal	CL	-	3	3
TOTAL			331	419	750
			44.13%	55.87%	100.00%

GE: Ecological Group; TOTAL sp.: Total individuals by species; NI: Not identified; CL: Light demanding climax; CS: Shadow tolerant climax; P: Pioneer. NI species were included in the sampling because it is possible to know which ecological group they belong to due to their position within the modules.

Figure 5 shows the survival and mortality rates of the seedlings by ecological group and proportional to the total planted, in relation to their location in the terrain (slope or plain). It was observed that the “CL” and “P” groups had a higher survival rate for the hillside seedlings, while the “CS” group had high survival rates at both locations.

Regarding the location of the modules in the field, the survival rates of the seedlings were lower in the modules located in the plain (Figure 6). Area C was the exception, since the mortality rate was higher in most modules, all located on the slope.

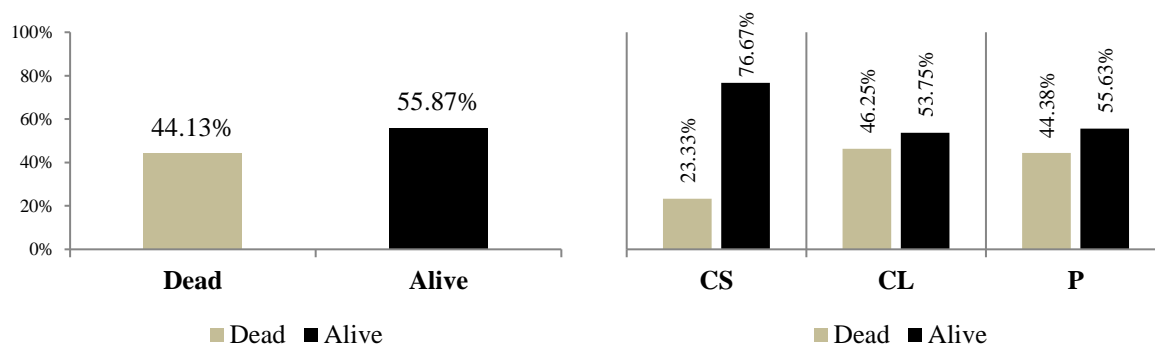


Figure 4. Mortality and survival of seedlings: at left, general aspect; at right, by ecological group (CL: Light demanding climax; CS: Shadow tolerant climax; P: Pioneer).

Figura 4. Mortalidade e sobrevivência das mudas: à esquerda, aspecto geral; à direita, por grupo ecológico (CL: Climática de luz; CS: Climática de sombra; P: Pioneira).

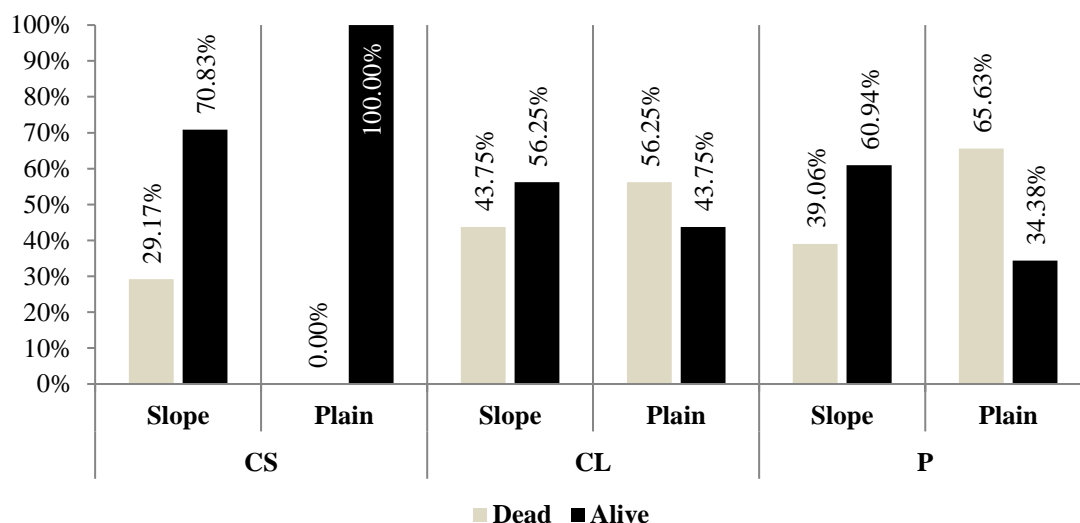


Figure 5. Survival and mortality of the seedlings by ecological group in relation to their location on the ground (CL: Light demanding climax; CS: Shadow tolerant climax; P: Pioneer).

Figura 5. Sobrevivência e mortalidade das mudas por grupo ecológico em relação à sua localização no terreno (CL: Climática de luz; CS: Climática de sombra; P: Pioneira).

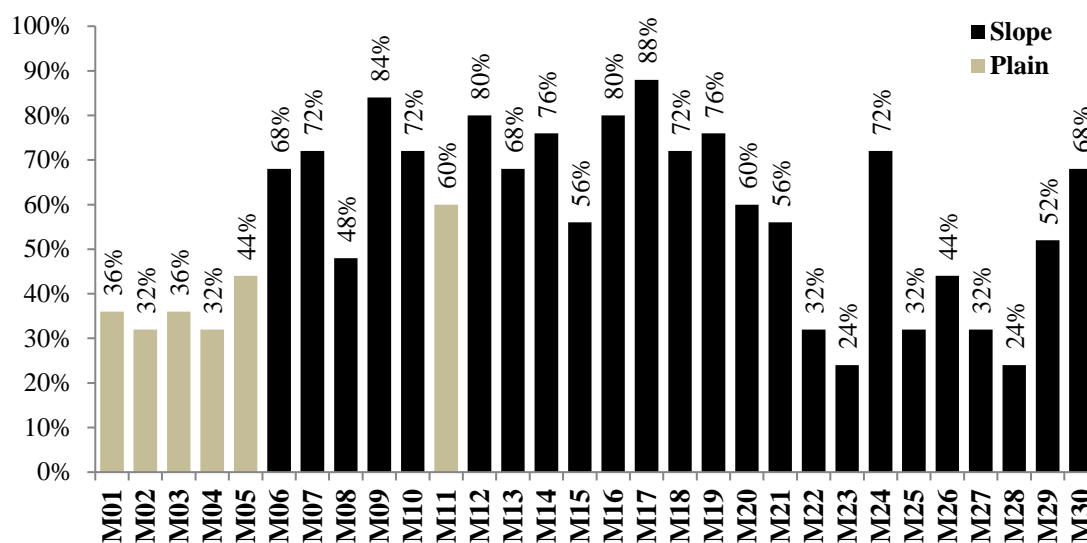


Figure 6. General survival of the seedlings by modules in relation to its location on the ground (M: Module).
 Figura 6. Sobrevivência geral das mudas por módulos em relação à localização no terreno (M: Módulo).

DISCUSSION

The use of labels printed on polyester films and affixed to the seedlings by means of polyamide plastic clamps proved to be a viable, low cost and practical alternative. Since they are made of inert material, with no attractive odor or flashy color, they have not attracted fauna, mainly small mammals, rodents and ants, which commonly attack field identification materials. Also, they did not undergo alterations provoked by the climatic conditions, like the heat, the humidity of the air and the precipitation, being kept intact and legible.

Other advantages of using this method are the possibility of individual customization of each label through texts or specific codes (alphabetical, numerical, alphanumeric, bar, two-dimensional bar code – known as QR or QR-code –, mixture of codes, among others), and any general software can be used for this purpose. Besides, determining the size of each label can be made according to its intended purpose. If barcodes or QR codes are used on labels, field inventories or surveys can be streamlined and facilitated with the use of portable reading and data entry devices for these codes. In addition, this material also accepts notes made in pencil or pen, which can be made in the field, if necessary. The unit cost was estimated at R\$ 0.17.

In the monitoring, it was possible to observe that the occurrence of frost affected 100% of the seedlings. This does not mean, however, that they died, since all the seedlings that survived showed advanced signs of regrowth, both of leaves and new stems.

The concern with the number of species planted in degraded areas came from the observation of the low wealth used in the older projects (BRANCALION *et al.*, 2010; MARTINS *et al.*, 2015). This fact is debated by several Brazilian researchers in ecological restoration projects that discuss the benefits of minimal restoration wealth (ARONSON, 2010; DURIGAN *et al.*, 2010; ARONSON *et al.*, 2011; MARTINS *et al.*, 2015). The greater the wealth, the greater the genetic diversity, which favors the adaptation to the most varied environmental situations (REIS *et al.*, 2003) since they are not related. Thus, a practice used in traditional plantations is avoided, in which seedlings from very distant areas are often used, entailing the entry of seedlings with genetic material very different from the typical species of the area to be restored (TRES; REIS, 2009).

The diversity of the surviving species of the seedlings used and evaluated in this study (Table 1) aligns with the concepts defended by the aforementioned authors. Besides, it favors the creation of an environmental heterogeneity, providing the formation of different niches and microhabitats and providing food and refuge for wildlife all year round (BARBOSA *et al.*, 2015).

The overall survival rate of the seedlings (Figure 4 on the left) of 55.87%, especially the “P” (55.63%) and the “CL” (53.75%), in this case the regionalization hypothesis, which is based on the premise that seeds of a local population are adapted to their conditions and, therefore, will result in greater success in restoration measures (BRANCALION *et al.*, 2009; BUSATO *et al.*, 2015).

Some studies, however, present the hypothesis that non-local genotypes introduced in a given area have a higher adaptive value than the ecotypes themselves (SALTONSTALL, 2002; PETIT, 2004). This phenomenon, called “cryptic invasion”, is based on the fact that local genetic materials are not always the best adapted to the biotic and abiotic conditions present in their region of occurrence (CRESPI, 2000). Thus, it contradicts the common

idea that local populations are always the ones with the greatest potential for adaptation to the environmental conditions where they occur (BRANCALION *et al.*, 2009), or even the idea that a lot of seedlings produced with local seeds will not necessarily represent the genotype that would be favored in the natural regeneration condition (BUSATO *et al.*, 2015).

The data presented in this study, however, demonstrate that the genetic material collected around the degraded area is well adapted to the climatic conditions of the region (regionalization hypothesis), because the forest matrix of this environment presents characteristics of resilience, since they are treated of forest areas in an advanced stage of succession. The cryptic invasion hypothesis seems to make more sense in areas that have an environment matrix with little or no resilience.

Therefore, it makes more sense, from the point of view of ecological processes and natural selection, to use propagules that have a high adaptive potential in the restoration projects, which can be obtained by seedlings of seeds generated from as many as possible of forest fragments. Thus, it would resemble what would be a natural process of gene flow between populations (BUSATO *et al.*, 2015).

Studies indicate that the planting of seedlings in field of several forest species is limited during the winter in the Southern Region of Brazil, due to the occurrence of low temperatures, which favor the formation of frost. It should be taken into account that plants are more susceptible to environmental in the early stages of their development, making it necessary to take measures to protect the seedlings, especially in nurseries installed in these regions (DE MARCO *et al.*, 2014).

As the seedlings are more exposed to the cold caused by the low temperatures near the ground, their survival can also be influenced according to their arrangement in the terrain relief, as pointed out in the graphs of Figures 5 and 6. These results showed that the rates of survival were higher in the seedlings located in the sloping areas for the three ecological groups, since the cold air, as it is denser, tends to accumulate in the lower regions of the plains.

All three ecological groups presented higher survival rates on the slope (Figure 5), with the exception of “CS”, which presented 100% of surviving seedlings also in the lowlands. Thus, it demonstrates that this ecological group is more adapted to adverse temperature conditions than “CL” and “P”. This last ecological group showed the lowest survival rates.

Similarly, the graph of Figure 6 shows that survival was lower in the modules installed in the lowlands (M01, M02, M03, M04, M05 and M11). The modules from M21 to M30, all installed in the slope of area C, contradict this observation, since their survival rates were also lower, reaching 32% in modules M22 and M25 and 24% in modules M23 and M28. A possible explanation for this setback is the fact that this area has the orientation of its face more inclined than the other two areas and it is oriented towards the west, which made the frost remains active for a longer period in that place, since the sun takes longer to reach its surface.

CONCLUSION

- Labels and fasteners have proven to be an efficient, practical and inexpensive system for identification of seedlings in the field, and resistant to the action of the climate (heat, humidity and precipitation). Moreover, they do not suffer damage or attacks from the local fauna.
- Frost caused the defoliation of 100% of the seedlings selected for the study.
- Overall survival of 55.87% may be considered high due to the occurrence of seven episodes of frost.
- The general regrowth and survival index of 55.87% can be considered as a positive characteristic due to the adaptation of the germplasm to the negative extreme temperature conditions that occur in the region.
- The ecological group of shadow tolerant climax is the one that best adapts to the extreme negative temperature conditions that occur in the region.
- In general, sloping areas present greater seedling survival.
- Guidance on the face of exposure is a relevant factor to be taken into consideration in areas subject to severe winters.

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