

Soil Seed Bank in the Tropical Rainforest Inserted in Agricultural Matrix, Northeast Region of Brazil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JPGS, LCM, ALPF and RLCF conceived and designed the research. Authors JPGS, WBS and JELT participated in the collection of soil within the forest remnant. Authors JPGS, LCM, ALPF, RLCF, WBS and JELT analyzed the data, wrote and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This research aimed to evaluate under two conditions of luminosity the floristic composition of the seed bank the soil of a remnant of Atlantic Forest inserted in an agricultural matrix located in the northeastern region of Brazil. Sixty samples of superficial soil were collected, with a distance, of approximately, 50 meters. The samples were placed in polyethylene bags, identified and transported to the forest nursery of the Federal Rural University of Pernambuco where they were placed under two conditions of luminosity, full sun and 70% shading. Count of emerged seedlings was performed daily. They were considered as emerged after the opening of the protophyllum. At 120 days, the seedlings were removed and transplanted into polyethylene bags and left in beds and the soil was stirred to facilitate the germination of some viable seeds that might still exist. To compare the richness and number of seedlings emerged in the two light conditions, ANOVA was performed. There were 3,965 seedlings, belonging to 15 botanical families and 29 morphospecies.

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In full sun, 524 emerged and under shading 70%, 3,441 seedlings. Considering the richness and number of emerged seedlings, there was a significant difference in the F test ($P=0.05$), because it presented a higher mean for the analyzed variables, the 70% shade presented better results, providing better conditions for seedling emergence. The seed bank as a potential source to produce seedlings of shrub-tree/tree-tree species, as they are responsible for the propagation of species of plant species, for the most part, for example, the beginning of degraded succession or colonization of clearings, with the worst result being under shading 70%.

Keywords: Seed bank; natural regeneration mechanism; forest restoration; agricultural matrix.

1. INTRODUCTION

The seed bank can be defined as the stock of viable seeds existing in the soil at a given moment, found from the surface to the deeper layers, whose seeds under adequate conditions can germinate and form the seedling bank [1,2]. This process is dynamic, varying according to input (rain and seed dispersal) and seed output (seed germination, predation and death).

In forest ecosystems, the seed bank is involved in some processes at the population and community levels, among them: establishment of populations; maintenance of species diversity; establishment of ecological groups; and restoration of species richness during natural forest regeneration [3].

Some factors can influence the composition of the seed bank, among them: land use history, the matrix in which the fragment is inserted, border effect, light intensity and vegetation composition [4,5]. Thus, the bank can be represented by seeds of the local vegetation and by seeds brought from more distant areas by means of the dispersion.

The study of the seed bank is related to different objectives, such as: potential and indicator of recovery of degraded areas; successional stage of vegetation; spatio-temporal variation; history of disturbance and others [6,7,8,9,10], providing information on the richness and floristic composition that will compose the vegetation in the future [2].

The seed bank along with litter transposition was used as efficient techniques, in forest restoration, to rescue the integrity of ecosystems in a way that can guarantee their sustainability [2,11]. Since during transposition of soil portions of preserved fragments to restore degraded areas, in addition to seeds, it transports microorganisms that are critical in nutrient cycling, soil restructuring and fertilization [12,13].

Studies on seed banks in the Atlantic Forest were carried out in several regions of Brazil, however, most of them were developed in the South and Southeast regions mainly in vegetation formations associated to the Atlantic Forest, including Semideciduous Seasonal Forest, Deciduous Seasonal Forest and Mixed Ombrophilous Forest [2,7,14,15,16]. Few studies have been carried out in Dense Ombrophilous Forest [10,17], being these vegetative formations of the Atlantic Forest. In the Northeast studies on seed bank in the Atlantic Forest are still scarce.

In view of the above, despite the advances in research, it is considered essential and of fundamental importance, the development of studies in all regions of Brazil that involve natural regeneration mechanism, such as seed bank evaluation, in a way that can generate knowledge of how a certain ecosystem is responding to the actions of the surroundings and yet what the possibility of serving as source of propagules for the restoration of degraded areas.

Therefore, this research aimed to evaluate under two conditions of luminosity the floristic composition of the seed bank the soil of a remnant of Atlantic Forest inserted in an agricultural matrix located in the northeastern region of Brazil.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is in a remnant of 243 hectares of the Atlantic Forest, Tropical Rainforest, where coordinates are 07°56'10.9"S and 35°03'43.7"W, located in the municipality of São Lourenço da Mata on land belonging to the Factory Petribú A/S., approximately 30 km from the city of Recife, capital of Pernambuco, Brazil. The area is embedded in an array of sugarcane, surrounded by the construction of firebreaks and roads.

The municipality is in the metropolitan area of the city of Recife, with vegetation classified as Dense Ombrophylous Forest the climate of the region according to the classification of Köppen, is of type As', having characteristics of tropical rainy climate with dry summer [18]. The mean temperature 27°C and precipitation of the study period was 1,710 mm, with March, June and July being the months with the highest and October, November and December the months with the lowest precipitation [19].

The soils of the region are represented by the Ferralsols in the flat tops, being deep and well drained; by Acrisols on steep slopes, being little to moderately deep and well drained, and by the lowland Gleysols in the backs of narrow valleys, with organic soils and soaked [20,21].

2.2 Data Collection

Seed bank sampling was performed in the center of 60 of the 80 already existing plots, located at the edge and interior of the fragment, where the arboreal component and the regenerating stratum were sampled in the floristic survey and phytosociological analysis in studies developed by [22] for the tree component and [23] for the regenerating stratum. Individuals with Circumference at Breast Height – CBH ≥ 15 cm (measured at 1.30 meters from the ground), were considered as arboreal. And, in regeneration, individuals with height ≥ 1 m and CBH < 15 cm.

The plots with dimensions of 10 m x 25 m were allocated systematically, equidistant 25 m, these being distributed in two parallel lines and each line with 10 plots. Being 40 plots on the edges of the fragment, 20 on each side and 20 plots in the interior, totaling 60 plots. The plots of the interior, distanced, approximately 350 m from the parcels allocated at the edge, is last, being allocated the shores of the fragment.

2.3 Sampling of the Seed Bank in the Soil

For the evaluation of the seed bank in the soil, a sample of 50 cm x 50 cm x 5 cm (length x width x depth) of the topsoil was taken along with the organic blanket (freshly fallen branches and dried leaves were removed) center of each plot, with the aid of an iron template, totaling 60 samples, and a total sample area of 15 m². Each sample was about 50 meters apart.

The samples were collected once, in the month of January. They were placed in polyethylene

bags, identified with the parcel number, then transported to the forest nursery of the Forestry Science Department of the Federal Rural University of Pernambuco (FScD/FRUPE). Subsequently, they were removed from the sacks and placed in wooden boxes and listed according to the parcel number. If there were seeds of different species that required different luminous intensity and temperature to stimulate the germination, 30 samples were covered by shadow 70% and the others were exposed to sunlight and covered with filo. All samples were watered twice a day (morning and afternoon) except for rainy days.

The seedling counts were counted daily for a period of 6 months (February to July 2015) and all seedlings were enumerated with increasing numbers. They were considered as emerged after opening of the protophyllum. At 120 days, the seedlings were removed and transplanted to polyethylene bags and left in stands to develop and acquire a morphological differentiation that allowed a more reliable identification of the species of Melastomataceae and some *Cecropia pachystachya* (seedlings that were smaller than 5 cm approximately), were placed in benches at the greenhouse. After the seedlings were removed, the soil was revolved to facilitate the germination of some viable seeds that could still exist in the soil.

In the weekly count the emergence speed of the seedlings was obtained, considering all the samples located under shadow 70% (7.5 m²) separated from the samples that were exposed to the sun (7,5 m²).

Temperature data, relative humidity and precipitation (Fig. 1) were used from the Automatic Agroclimatic Station located in the Professor Dr, Ronaldo Freire de Moura's Irrigated Agriculture Station in the Department of Agricultural Engineering of the Federal Rural University of Pernambuco, located at a distance of approximately 300 m from the forest nursery.

2.3 Sampling of the Seed Bank in the Soil

During the soil collection, fruiting species were collected and seeds were placed in washed sand in a greenhouse in the forest nursery – FScD/FRUPE to germinate and assist in the identification of seedlings emerged in the seed bank. Individuals who presented themselves with flowers and fruits had collected botanical material. This material was duly herborized and

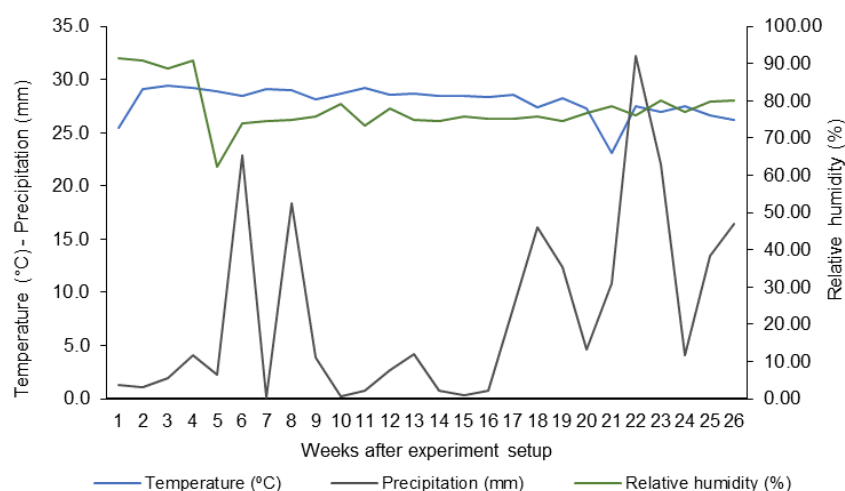


Fig. 1. Mean weekly temperature, humidity and precipitation

Source: Automatic Agroclimatic Station from the Professor Ronaldo Freire de Moura's Irrigated Agriculture Station from the Department of Agricultural Engineering.

identified by comparison with exsiccates at the Sérgio Tavares Herbarium (STH) - FScD/FRUPE, to assist in the identification. Literatures [22,23,24,25,26] and specialists were also consulted.

2.4 Data Analysis

Seedlings emerged on the soil seed bank were counted, separated into morphospecies and identified in families, gender and, where possible, in species, with scientific names and their respective authors, updated according to The International Plant Names Index database, through of the site <http://www.ipni.org>. The regional names were also annotated and classified as successional category and the dispersion syndrome.

As for the successional category in: pioneers, species highly dependent on light; secondary, occur in conditions of medium shading or not very intense luminosity; late development, develop in the sub-forest under light or dense and uncharacterized shade conditions, considering the species that do not have information about the ecological group to which they belong [27]. In the unclassified category were also included the seedlings or diaspores that were classified as morphospecies or that were identified at family and gender level.

For the classification of diasporic dispersion syndromes, the classification proposed by [28] was used in: anemochoric, species that have diaspores with dispersion by the wind; zoochoric,

species that have diaspores with dispersion by animals and autochoric that are the species that have diaspores with dispersion by the gravitational force or that have mechanisms of self-dispersion.

The experiment in a nursery was carried out in a completely randomized design (2 Treatments with 30 repetition each). In order to compare the seeds bank in the soil evaluated in the forest, under 70% shade and, in the full sun, considering the variables richness and number of emerged seedlings. The Analysis of Variance (ANOVA) was performed by the F test at a level of 95% of confidence. The data were analyzed using software Microsoft Excel for Windows™ 2013.

3. RESULTS AND DISCUSSION

3.1 Floristics, Wealth and Diversity

During the six months of observations, there were 3,965 seedlings of tree species belonging to 15 botanical families. Seeds of 29 morphospecies were germinated, 19 of which were identified at a specific level, 7 at a generic level, 1 at the family level and 2 could not be classified at any taxonomic level (Table 1). One of the greatest difficulties found in the present study was that morphospecies did not grow enough during the study period and did not present morphological characteristics for comparison with adult individuals and possible identification.

Table 1. Floristics, successional classification and dispersion syndrome of emerged seedlings from Atlantic forest's seed bank of shrub-tree/tree-trees species

Family / Species name	Shading 70%	Full sun	EG	DS
Annonaceae				
<i>Xylopia frutescens</i> Aubl.	X	x	Is	Zoo
Araliaceae				
<i>Schefflera morototoni</i> (Aubl.) Maguire, Steyerm. & Frodin	X	x	Is	Zoo
Burseraceae				
<i>Protium</i> sp.		x	Wc	Zoo
Cecropiaceae				
<i>Cecropia pachystachya</i> Trécul	x	x	Pi	Zoo
Clusiaceae				
<i>Vismia guianensis</i> (Aubl.) Pers.	x	x	Pi	Zoo
Euphorbiaceae				
<i>Pera glabrata</i> (Schott) Poepp. ex Baill.	x	x	Is	Aut
Fabaceae				
<i>Albizia pedicellaris</i> (DC.) L. Rico	x		Pi	Aut
<i>Bowdichia virgilioides</i> Kunth	x	x	Ls	Ane
<i>Parkia pendula</i> (Willd.) Benth. ex Walp.	x	x	Ls	Aut
<i>Sclerolobium densiflorum</i> Benth.	x		Wc	Ane
Fabaceae 1	x		Wc	Wc
Flacourtiaceae				
<i>Casearia sylvestris</i> Sw.	x		Is	Zoo
Lauraceae				
<i>Ocotea glomerata</i> (Nees) Mez	x	x	Is	Aut
Malpighiaceae				
<i>Byrsonima sericea</i> DC.	x	x	Is	Zoo
Malvaceae				
<i>Apeiba albiflora</i> Ducke	x	x	Pi	Aut
Melastomataceae				
<i>Miconia affinis</i> DC.	x	x	Is	Zoo
<i>Miconia</i> cf. <i>hypoleuca</i> (Benth.) Triana	x	x	Is	Zoo
<i>Miconia minutiflora</i> (Bonpl.) DC.	x	x	Is	Zoo
<i>Miconia prasina</i> (Sw.) DC.	x	x	Pi	Zoo
<i>Miconia</i> sp. 1*	x	x	Wc	Zoo
<i>Miconia</i> sp. 2	x	x	Wc	Zoo
<i>Miconia</i> sp. 3	x	x	Is	Zoo
<i>Miconia</i> sp. 4	x		Wc	Zoo
<i>Miconia</i> sp. 5	x		Wc	Zoo
Moraceae				
<i>Ficus</i> sp.	x		Wc	Zoo
Siparunaceae				
<i>Siparuna guianensis</i> Aubl.	x		Is	Zoo
Ulmaceae				
<i>Trema micrantha</i> (L.) Blume	x		Pi	Zoo
Morphospecies				
Morphospecies 1	x		Wc	Wc
Morphospecies 2	x	x	Wc	Wc

In which: EG = Ecological group; Pi = Pioneer; Is = Initial secondary; Ls = Late secondary; Wc = without characterization; DS = Dispersion Syndrome; Zoo = Zoochoric; Ane = Anemochoric; Aut = Autochoric; Wc = without characterization.

* *Miconia* sp. 1 includes all individuals that were identified at the gender level, but which died before the possible identification at the species level.

In the samples located in full sun were recorded, 523 emerged seedlings, distributed in 11 families and 19 morphospecies, of which 14 at specific level, 4 at generic level, 1 remained unidentified.

In the samples placed under shading 70%, 14 botanical families were identified and 3,441 emerged seedlings, distributed in 28 morphospecies, of which 19 were identified at the specific level, 6 at a generic level, 1 in the family and 2 without identification.

The similarity between the floristic composition and the Sørensen index was 0.77, having a similar composition, however, considering the richness and number of seedlings emerged by the Analysis of Variance (ANOVA), there was a significant difference by the F test ($P = .05$) between the seedlings emerged under 70% shading and in full sun, due to the higher average for analyzed variables, 70% shade presented better results, providing better conditions for emergence of the seedlings.

These differences have also been observed in other studies evaluating seed bank under different shading conditions in other phytogeographies of the country, corroborating with the results obtained by [29] that evaluated the seed bank in a semideciduous seasonal forest area, Pinheiral - RJ (15% and 70% shading) showed the best results in 70% of shading, where 3,940 seeds were germinated and 613 were trees. Comparing the number of tree species, it differed from the results found by [30] that evaluated the seed bank in a seasonal semideciduous forest in Viçosa-MG, southeastern region of Brazil, of the 3,416 seedlings registered in the seed bank, 1,390, germinated under shading of 11.5% of which 448 were tree species, and 2,026, under 60% shading, being 368 tree species, with no statistically significant differences being observed. These results (full sun and 70% shading) show that shading can provide better conditions and support the germination of the seeds contained in the soil.

In this writing, the highest number of seedlings emerged below 70% of shade may have been a consequence of the more favorable conditions offered, with the level of light required for the germination of these species and the humid environment, which prevented soil dryness, all samples were watered twice a day. With higher solar incidence and higher temperature which the samples in full sun were exposed, may have hindered the germination of the species.

The species of the families Salicaceae, Siparunaceae, Moraceae and Ulmaceae germinated only in the seed bank in shading

70%. The most abundant botanical families, considering all the sample units, were Melastomataceae (9) and Fabaceae (5), other families presented only one species each.

Melastomataceae and Fabaceae presented greater richness, in tree vegetation sampling, for the adult and regenerating strata [21,22]. The high representativity of Melastomataceae, especially *Miconia*, has also been observed in other studies of seed bank in the soil [16,17,29,31]. Its presence can be influenced not only by established vegetation but also by nearby fragments brought by dispersing agents, being essential its presence in the seed bank for the reestablishment of the area with the emergence of clearings.

In relation to the richness of arboreal species identified in the seed bank the value found in the present study has been higher than those found by some authors evaluating seed bank in the same forest typology [10,17]. This difference may be related to the location and time of soil collection for evaluation, established vegetation, as well as the age of the forest. For there is a positive correlation between the age of the forest and the richness of tree species in the seed bank [32].

Among the emerged seedlings that were identified at the specific level, *Trema micrantha* was the only species that was not part of the floristic composition of the tree vegetation [21, 22]. In Brazil, *Trema micrantha* commonly occurs as an abundant species of seed bank studies [4, 10, 33, 34], because it is a pioneer species and have dormant seeds [35]. They can remain dormant in the soil for long periods and with the appearance of a clearing, they can germinate, if provided favorable conditions of temperature, luminosity and humidity.

Some seeds contained in the seed bank require a rupture of the litter layer or some disturbance in the soil to germinate, for example, the fall of a tree that provokes beyond the opening of the canopy the soil revolving [36]. Pioneering species such as *Trema micrantha*, *Cecropia pachystachya* and other pioneers found in this study will be the first species to germinate if they are in the soil seed bank and when adults can favor the arrival of other species.

For emergence speed of seedlings, in the soil seed bank, located under 70% shading, a total of 411 and 554 emerged seedlings were observed,

at the 8th and 26th week, with a rate of 51.4 and 21.3 seedlings/week, 7.5m^{-2} respectively. This was differentiated from the seed bank located in full sun, which was obtained at the highest rate, 7.23 seedlings/week, equivalent to 188 germinated seedlings, occurred at the 26th week (Fig. 2).

The first tree species to emerge after the installation of the experiment were *Albizia pedicellaris* and *Trema micrantha* in the first and second week respectively, in shading 70%; already in full sun the first emergency occurred in the seventh week with the species *Cecropia pachystachya*, *Byrsonima sericea*, *Bowdichia virgilioides* and *Parkia pendula*. Being *Cecropia pachystachya* and *Miconia prasina* the species that had the highest number of seedlings emerged under both conditions, full sun and under 70% shading. This difference in seed germination and in the number of emerged seedlings may have been due to higher humidity and lower soil temperature, favored by the greater precipitation occurred in the months of March, June and July, and may still be associated with the type of dormancy, as primary or secondary [37].

3.2 Succession Classification and Dispersion Syndrome

In the successional classification there was a predominance of the initial secondary species followed by the pioneers (Table 2). The presence of arboreal species classified as pioneer, early secondary and late, in the seed bank, indicates a high potential for regeneration of the tree component, in the case of a clearing in the forest structure, as they provide the beginning and the necessary support for the advancement of dynamics [14,38].

From the total number of species recorded, there was predominance of species with zoochoric dispersion, followed by autochoric and anemochoric dispersion (Table 3). Zoochoric dispersion has predominated in woody species, ranging from 52.9% to 98.7% [39, 40] and in other seed bank studies [15, 17], thus demonstrating the importance of the presence of frugivorous animals within the fragment for contribution in the secondary dispersion and in the dynamics within the fragment.

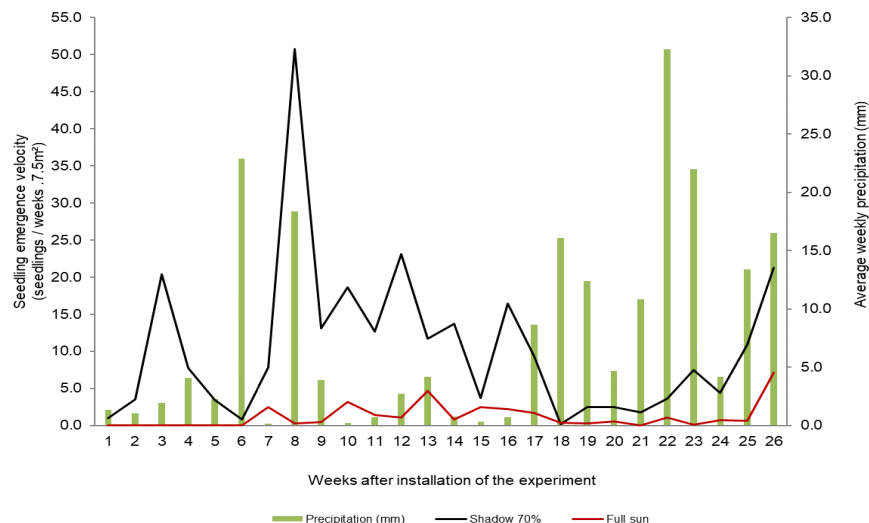


Fig. 2. Emergence of seedlings of shrub-tree/tree-tree species identified in the Atlantic Forest seed bank

Table 2. Classification of the ecological group of tree species found in the soil seed bank

Category	General	Shading 70%	Full Sun
Secondary Initial	37.9	39.3	47
Pioneer	20.7	21.4	21
Late Secondary	6.9	7.1	11
Without classification	34.5	32.2	21

Table 3. Seeds dispersion syndrome of the shrub-tree/tree species identified in the seed bank.

Dispersion syndrome	General	Shading 70%	Full Sun
Zoochoric	65.5	64.3	68.4
Autochoric	17.2	17.9	21.0
Anemochoric	6.9	7.1	5.3
Without classification	10.4	10.7	5.3

3.3 Density of Tree Species in the Seed Bank on the Soil in a Forest Nursery

The average seed density of tree species for samples placed under 70% shade was 458.8 (\pm 98.82) and 69.73 (\pm 26.90) seeds/m² for the full sun; considering all sample units, the mean density was 266.6 seeds / m².

The average density of emerged seedlings of recorded tree species differed from the values found by other authors when evaluating seed bank germination in Dense Ombrophylous Forest Fragment with 222.59 [10] and 1,308.9 seeds.m⁻² [17]. The seed matrix composition and its density, among them: land use history, matrix in which the fragment is inserted, light intensity and vegetation composition [4,5].

The five species with the highest density were: *Miconia prasinia*, *Cecropia pachystachya*, *Miconia* cf. *hypoleuca*, *Miconia affinis* and *Miconia* sp.3 (shading 70%) and *Cecropia pachystachya*, *Miconia prasinia*, *Apeiba albiflora*, *Miconia* sp.2 and *Byrsonima sericea* (full sun), these species will probably be the first to appear in the occurrence of some disturbance in the area.

4. CONCLUSION

The seed bank of the remnant showed potential for reestablishment in the occurrence of disturbances because it presented in its richness, species categorized in the different ecological groups. Mostly composed of species from the beginning of the succession, which are responsible for the colonization of areas previously degraded or by colonization of clearings.

To use seedlings from the seed bank in restoration of nearby degraded areas or with the same phytophysiognomy. Species such as *Miconia prasinia*, *Cecropia pachystachya*, *Apeiba albiflora*, and *Byrsonima sericea* can be used because they were common and abundant in the treatments full sun and shading 70 %.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Analysis of the Variety of Variables: number of seedlings emerged and wealth for the seed bank, evaluated in a forest nursery.

Variance Analysis of the Wealth Variable						
Source of variation	SQ	gl	MQ	F	F critical	P
Between groups	493,07	1	493,09	160,42	4,001	2,41E-18
Among the groups	178,27	58	3,07			
Total	671,33	59				

Variance analysis of the number of emerged seedlings						
Source of variation	SQ	gl	MQ	F	F critical	p
Between groups	141912,1	1	141912,07	60,272	4,01	1,53E-10
Among the groups	136561,7	58	2354,51			
Total	278473,7	59				

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