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Chronological analysis of the physiological quality of diaspores of *Myracrodruon urundeuva* Fr. All. in semiarid regions

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ABSTRACT. The objective of this study was to evaluate the physiological quality of diaspores from *Myracrodruon urundeuva* mother trees developing in the Cariri region of the state of Paraíba, Brazil. The seeds were collected during three consecutive years and from different areas of the Cariri region. The seed collection areas comprised two sites in the municipality of Boa Vista and one in the municipality of São João do Cariri. The diaspores had their water content determined and were submitted to the following tests: germination, seedling emergence, first count of germination and seedling emergence (within 5 days), and speed of germination and seedling emergence. The treatment means were compared using the deviance statistic. The collection years and collection areas were compared by the Tukey test at a 5% probability level. The comparison between mother trees was made through principal component analysis and cluster analysis. The detected variation in the quality of *M. urundeuva* diaspores is dependent on environmental conditions. The physiological quality of *M. urundeuva* diaspores varies according to mother tree, collection area and year. Mother trees of groups 1 and 2 are the most promising since they included representatives of all three areas and produced diaspores of high physiological quality.

Keywords: aroeira; physiological quality; seeds; Caatinga.

Análise temporal da qualidade fisiológica de diásporos de *Myracrodruon urundeuva* Fr. All. em regiões semiáridas

RESUMO. Neste estudo objetivou-se analisar a qualidade fisiológica de diásporos de matrizes de *Myracrodruon urundeuva*, em três anos de coletas e diferentes áreas do Cariri paraibano, Brasil. Uma área no município de São João do Cariri, e duas em Boa Vista foram demarcadas, cujas matrizes foram avaliadas pela viabilidade e vigor de seus diásporos em três anos consecutivos. Diásporos das matrizes selecionadas foram submetidos à determinação do teor de água e as seguintes variáveis: germinação, emergência de plântulas, primeira contagem de germinação e de emergência de plântulas, índice de velocidade de germinação e de emergência de plântulas, índice de velocidade de germinação e de emergência de plântulas. A significância dos efeitos foi realizada através da estatística deviance. Para os fatores anos e áreas de coleta as médias foram comparadas pelo teste de Tukey a 5% de probabilidade e para a variação entre as matrizes realizou-se análise de componentes principais e agrupamento. A variação da qualidade fisiológica de diásporos de *M. urundeuva* é dependente dos fatores ambientais. A qualidade fisiológica de diásporos de *M. urundeuva* soria em relação às matrizes, áreas e anos de coleta. As matrizes dos grupos 1 e 2 são as mais promissoras por conterem representantes das três áreas e produzirem diásporos com elevado potencial fisiológico.

Palavras-chave: aroeira; qualidade fisiológica; sementes; Caatinga.

Introduction

The Caatinga, a biome in the Northeast Region of Brazil, typically has a semiarid climate with plants physiologically adapted to extreme environmental conditions such as high temperatures, high radiation levels and evaporation; this specialization is an important reason for their high rate of endemism (Berger et al., 2007). However, the characteristics of the plants occurring in this biome are not fully known, and this complicates research efforts, especially those taking place at the phase of selection, owing to the low number of individuals and the need to collect winged diaspores.

Myracrodruon urundeuva Fr. All. is a perennial species of the Anacardiaceae family, native to Brazil and occurring abundantly in the Caatinga

region, where it is also known as "aroeira", "aroeirado-sertâo", "aroeira-do-cerrado", and "aroeira-preta" (Lorenzi, 2008; Pereira, Barros, Brito, Duarte, & Maia, 2014).

Since it has the characteristics of pioneer species (a deep root system and adaptation to adverse soil and climate conditions), M. urundeuva is recommended for the recuperation of degraded ecosystems, reforestation, and economic wood exploitation (Lorenzi, 2008; Kratka & Correia, 2015; Mota, Silva, Souza, Oliveira, & Santos, 2015; Canuto, Silva, Moraes, & Resende, 2016). In addition, the presence of phytochemical compounds in its bark, such as natural dimeric chalcones, which are anti-inflammatory products, and urundevein A and B, indicates the pharmaceutical potential of this species (Souza et al., 2007; Carlini, Duarte-Almeida, Rodrigues, & Tabach, 2010; Pereira et al., 2014; Machado, Sartori, Damante, Dokkedal, & Oliveira, 2016).

Owing to its social and economic importance, M. urundeuva has been subject to years of intense and predatory exploitation, which has led to a devastation of its natural populations (Canuto et al., 2016). Therefore, in order to administer programs for reforestation and the preservation of genetic resources, it is necessary to expand our knowledge about the germination and propagation of M. urundeuva, since the currently available information about the physiological quality of its seeds is not conclusive (Virgens, Castro, Fernandez, & Pelacani, 2012; Diniz, Diniz, Azeredo, Souza, & Pereira, 2015).

To produce representative plantlets and to plant thickets for reforestation or production, it is necessary to work with thoroughly mixed seeds representing at least 12 to 13 mother trees. However, studies concerning the variability among mother trees in terms of seed germination are relatively scarce (Santos, Paula, Sabonaro, & Valadares, 2009; Souza et al., 2015), necessitating new research works to obtain more practical results.

On the other hand, the selection of individual plants with desirable performance characteristics may be carried out early on the basis of seed physiological quality (Martins-Corder & Saldanha, 2006), as determined by seedling emergence and vigor tests. This enables the production of plantlets on more efficient commercial scales by reducing mean germination time and increasing emergence uniformity and seedling size (Martins, Bovi, Oliveira, & Vieira, 2013). Thus, the objective of this project was to evaluate the physiological quality of *M. urundeuva* diaspores collected from different mother trees during three years in different areas of the Cariri region in the State of Paraíba, Brazil.

Material and method

This study was carried out with *M. urundeuva* diaspores collected in the municipalities of São João do Cariri and Boa Vista, in the Brazilian State of Paraíba. In the municipality of Boa Vista, the diaspores were collected from two areas.

The municipality of São João do Cariri is located in the Eastern Cariri microregion at the geographical coordinates of 7°22'45.1"S and 36°31'47.2"W, with an altitude of 400 to 600 m above sea level and a semiarid BSh climate. The locale has a mean annual rainfall of 400 mm, a mean minimum temperature of 22.1°C and a mean maximum of 27.2°C; the mean relative humidity is 70% (Köppen & Geiger, 1928; IBGE, 2015).

The municipality of Boa Vista is located in the Campina Grande microregion at the geographical coordinates of 7°15′34″S and 36°14′24″W, with a mean altitude of 493 m above sea level, and the climate of the region is semiarid, classified as BSh. The locale has a mean yearly precipitation of 500 mm, a mean minimum temperature of 16.8°C, and a mean maximum of 29.2°C; the mean relative humidity is 65% (Köppen & Geiger, 1928; IBGE, 2015).

M. urundeuva mother trees were selected and marked using the trail method as the sampling criterion. This strategy is viewed as optimal for achieving the highest possible number of mother trees, which are distributed in all areas. The trees were selected considering the size of the *M. urundeuva* population the areas, as well as canopy formation and sanitary appearance; the selected trees were georeferenced using GPS and identified with wood pickets and satin ribbons.

In each area, mature diaspores from the selected mother trees were collected between September and December of the three consecutive years of the experiment (2012, 2013, and 2014). However, owing to the characteristic temporal and spatial variation in the production of diaspores by this species, the number of mother trees varied between areas in the last two years – in the final two years, at the time the observations were being made, some of the trees had produced few or no diaspores, thus making it impossible to collect any diaspores (Table 1).

Table 1. Distribution of the Myracrodruon urundeuva mother trees

 selected for diaspore collection during three consecutive years in

 the municipalities of São João do Cariri and Boa Vista in the State

 of Paraíba, Brazil.

A	Mathan toos	Year			
Area	Mother tree —	1	2	3 X	
	1	Х		Х	
	2	Х	Х	Х	
São João do Cariri	3	Х	Х		
Sao Joao do Carin	4	Х	Х	Х	
	5	Х	Х	Х	
	6	Х		Х	
	7	Х	Х	Х	
	8	Х	Х	Х	
	9	Х	Х	Х	
Boa Vista (Area 1)	10	Х	Х		
	11	Х	Х	Х	
	12	Х	Х	Х	
	13	Х			
	14	Х			
	15	Х			
	16	Х	Х	Х	
	17	Х	Х	Х	
	18	Х		Х	
	19	Х			
	20	Х	Х	Х	
Boa Vista (Area 2)	21	Х			
	22	Х			
	23	Х	Х	Х	
	24	Х			
	25	Х			
	26	Х	Х		
	27	Х		Х	
	28	Х			

X indicates mother trees from which no diaspores were collected.

Each year, the collected diaspores were transported in thermic boxes to the Seed Analysis Laboratory of the Plant Production and Environmental Sciences Department of the Federal University of Paraíba (UFPB), located in Areia in the state of Paraíba; at the laboratory, the diaspores were submitted to processing for the removal of the wings. Subsequently, the water content of the diaspores was determined by drying them in an oven at $105 \pm 3^{\circ}$ C for 24 hours (Brasil, 2009). For that measurement, four replicates of 20 diaspores each were used.

Before being submitted to the germination test, the diaspores were disinfested with sodium hypochlorite at a concentration of 0.5% for 5 minutes. Subsequently, four sets of 25 diaspores per individual sample were sown in $11.0 \ge 11.0 \ge 3.5$ cm transparent plastic boxes in a commercial vermiculite substratum with a mean granulometry and a water retention capacity of 60%. Then, the diaspores were placed in a germination chamber at a constant temperature of 25°C. Counts were taken five and ten days after sowing (Pacheco, Matos, Ferreira, Feliciano, & Pinto, 2006).

Seed vigor was evaluated by means of the following tests:

Seedling emergence in sand – the test was conducted in a protected environment with a semicircular electroplated steel roof covered with a polyethylene film and the sides covered with thin plastic mesh. Four samples of 25 diaspores were submitted to the disinfestation procedure previously described. Then, they were sown at a depth of 1 cm in washed and sterilized sand. Seedling counts were taken on the fifth and tenth days after sowing. Any seedling with its hypocotyl loop extending above the substratum level was considered an emerged seedling.

First count of germination and seedling emergence – this measure was defined as the total number of seedlings emerged by the fifth day after sowing.

Speed of germination and seedling emergence indexes – these measures were determined according to the equation proposed by Maguire (1962).

Treatment replications were distributed according to a completely random design. For data analysis, general linear models were used, assuming the beta distribution for germination, emergence and first count of germination percentages and a lognormal distribution for the speed indexes, considering collection years and loci. The comparison between means was made by the Tukey test at a 5% probability level. Subsequently, principal component analysis and cluster analysis were performed.

Result and discussion

The evaluated *M. urundeuva* diaspores showed low initial water content variation between samples from the different areas and years (2012, 2013, and 2014: 10.6 \pm 0.6, 10.7 \pm 0.6, and 11.5 \pm 1.2%, respectively). This is an important fact in the evaluation of diaspore quality, since the uniformity of water content is indispensable for standardizing these evaluations and achieving trustworthy results (Marcos Filho, 2015). Similar results were reported by Lucio, Homrich, and Storcki (2007) and Virgens et al. (2012) – initial diaspore water content of 10.1 and 9.6%, respectively.

The germination test, as well as the tests for the speed of germination index and first count of germination, showed that the interaction between area and collection year was not statistically significant. The other variables showed significant responses. When the isolated factors were considered, all variables had significant responses, except for germination and speed of germination in relation to the year factor (Table 2).

Table 2. Analysis of variance results for the germination test (G), first count of germination (FCG), speed of germination index (SGI), emergence (E), first count of emergence (FCE), and speed of emergence index (SEI) of *Myracrodruon urundeuva* diaspores collected during three consecutive years in the municipalities of Boa Vista and São João do Cariri, State of Paraíba, Brazil.

Source	Degrees	F values					
of variation	of freedom	%G	%FCG	SGI	%Е	%FCE	SEI
Area	2	25.23**	26.62**	19.80**	100.81**	61.49**	93.57**
Year	2	1.60 ^{ns}	2.96**	2.46 ^{ns}	6.87**	3.97*	10.11**
Area x Year	4	0.89 ^{ns}	9.20**	1.08 ^{ns}	4.60**	1.25 ^{ns}	6.63**
** significa	ntata 1% r	probability	level ($p \leq$	0.01)· * si	onificant at	a 5% prob	ability level

 $(0.01 > p \le 0.05);$ ^{ns} nonsignificant (p $\ge 0.05)$

It is important to emphasize that, in the last two years of observation, there was a reduction in the number of trees selected for the collection of diaspores (Table 1). This was due to the very low number of diaspores produced by some mother trees.

Among production factors, rainfall is one that acts by inducing physiological response mechanisms in plant. During the collection periods, seasonal variation of rain precipitation was verified; in the first year, the collecting of diaspores occurred under dry conditions, which were preceded by a rainy period that enabled adequate production of fruits by a larger number of mother trees (Figure 1).

The occurrence of phenological events in some species is not determined primarily by rain but by the level of water available to the plants (Borchert & Rivera, 2001). Therefore, during the dry season, when rain is scarce, the species, as a consequence of the end of the reproductive phenophase, express all their production of dry fruits, anemochorics, small seeds, orthodox (Griz & Machado, 2001; Ragusa-Netto & Silva, 2007; Silva, Prata, Mello, & Santos, 2013; Japiassú, Lopes, Dantas, & Nóbrega, 2016).

The rain precipitation pulses taking place in a given area increase the water level of the system. However, the amount of water that becomes available for use by plants depends on factors such as soil shallowness and proximity to rock formations that make difficult water penetration in the soil profile. Therefore, the production of leaves and fruits is dependent on the amount of available water; by analogy, variability is expected among different mother trees of the same species (Andrade, Souza, Silva, Silva, & Lima, 2006).

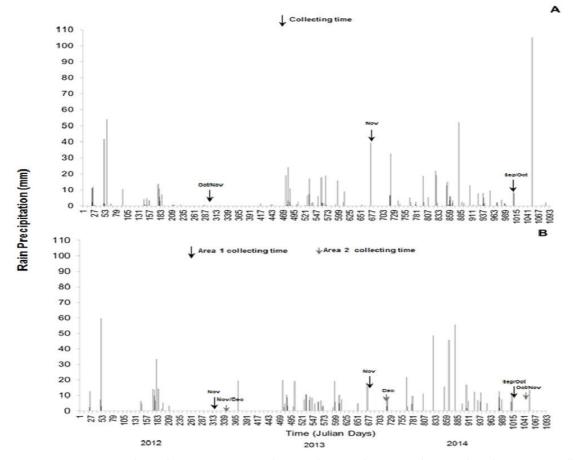


Figure 1. Rain precipitation during the years 2012, 2013, and 2014 in the areas where *Myracrodruon urundeuva* diaspores were collected in the municipalities of São João do Cariri (A) and Boa Vista (B), in the state of Paraíba, Brazil.

In addition to those aspects, Santos, Vieira, Fagundes, Nunes, and Gusmão (2007) report that, within the same forest species, individual variations among trees can be found, these differences being due to environmental influences during seed development and to genetic variability; their report is in agreement with the results reported herein. According to the aforementioned authors, these factors have been mentioned as the main causes of the variations in the germination process. Similarly, Lima, Bruno, Silva, Pacheco, and Alves (2014) report variations in seed physiological quality in Poincianella pyramidalis (Tul.) L.P. Queiroz (Fabaceae) mother plants growing in the same collection area.

The germination rate of the collected *M. urundeuva* diaspores varied between 4% and 93%. A similarly large variation (between 16% and 90%) was also reported by (Dorneles, Ranal, & Santana, 2005; Pacheco et al., 2006; Nunes, Fagundes, Almeida, & Veloso, 2008; Guedes et al., 2011; Virgens et al., 2012; Scalon, Scalon Filho, & Masetto, 2012).

As shown by the first count data, the germination rate of *M. urundeuva* diaspores also varied with location and year of collection (Table 3). The diaspores collected from the Boa Vista area in the first and second years exhibited the highest first count values, although the seeds did not significantly differ in this respect from the seeds collected in the São João do Cariri area.

Table 3. First count of germination, emergence and speed of emergence index of *Myracrodruon urundeuva* plantlets from diaspores collected during three consecutive years in the municipalities of Boa Vista and São João do Cariri, state of Paraíba, Brazil.

	First count of	First count of germination (%) (mean ± standard		
Area		deviation)		
	1 st year	2 nd year	3 rd year	
São João do Cariri	49 ± 23 aA	14 ± 7 bB	17 ± 18 aB	
Boa Vista – Area 1	$46 \pm 24 \text{ aA}$	38 ± 14 aA	19 ± 12 aB	
Boa Vista – Area 2	$33 \pm 21 \text{ bB}$	48 ± 26 aA	7 ± 6 aC	
	Emergence (mean \pm standard deviation)			
São João do Cariri	63 ± 14 abA	22 ± 12 aC	44 ± 14 aB	
Boa Vista – Area 1	65 ± 17 aA	$28 \pm 15 \text{ aB}$	$20 \pm 11 \text{ bB}$	
Boa Vista – Area 2	$56 \pm 22 \text{ bA}$	17 ± 13 aB	$16 \pm 12 \mathrm{bB}$	
	Speed of eme	rgence index (mea	n ± standard	
	deviat	ion)		
São João do Cariri	3,42 ± 1,20 abA	$1,14 \pm 0,70 \mathrm{bC}$	1,94 ± 0,69 aB	
Boa Vista – Area 1	3,49 ± 1,03 aA	$1,82 \pm 1,01 \text{ aB}$	$0,92 \pm 0,45 \mathrm{bC}$	
Boa Vista – Area 2	2,81 ± 1,21 bA	$0.97 \pm 0.82 \text{bB}$	$0,64 \pm 0,48 \mathrm{bB}$	

Means in the same column followed by the same lowercase letter and means in the same row followed by the same capital letter are not significantly different according to the Tukey test ($p \le 0.05$).

The first count test results are an indication of the speed of germination. Variation in the speed of germination is caused by differences in the physiological potential of the diaspores, since, according to Nunes et al. (2008), *M. urundeuva* diaspores are not dormant and the speed of

germination depends on seed coat permeability, air temperature, and seed chemical composition.

The low germination rate of the diaspores collected in the third year is probably due to the rain precipitation taking place during diaspore dispersion (Figure 1); this aspect was highlighted by Griz & Machado (2001) in their year-long study of the mode of seed dispersion during the rainy and dry seasons, as well as the growth habits and fruit morphology, of 42 species inhabiting the semiarid region of Northeast Brazil, including *M. urundeuva*.

The seedling emergence and speed of emergence index results showed that the diaspores collected in São João do Cariri and area 1 of the Boa Vista farm (Table 3) were of high quality, although the results were not significantly different from those of seeds collected at area 2 of Boa Vista and at São João do Cariri. This is an indication that the diaspores used in this work were of good quality, independently of the year and place of production. This fact may be justified by the occurrence of different rain precipitation conditions that influenced the diaspores maturation process and was verified in the germination and seedling emergence tests (Table 3). In addition, it is necessary to consider the particular environmental factors taking place at the location of each mother tree, such as the amount of available water, shallow soil conditions and the elevation of rocky formations, as well as the genetic variation among mother trees.

The results permit us to visualize the variations among the evaluated individuals. According to Diniz et al. (2015), it is possible to verify, within the same species, individual variations caused by environmental conditions prevailing during seed development and by genetic variability. These variations may directly affect seed germination and seedling development.

As a general rule, the final number of germinated diaspores and/or of emerged seedlings is considered important information for seedling producers. This information is usually gathered in vigor tests that also include the commonly used speed index, since germination and emergence rates are considered a more accurate measurement of the real physiological condition of seeds (Dorneles et al., 2005).

According to Virgens et al. (2012), the speed of emergence index (SEI) of a given seed lot is an important tool to define the speed with which a new forest community could be occupied. Index values close to those herein reported were reported by Guedes et al. (2012), with values between 1 and 3 in *M. urundeuva* diaspores sown

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under the same substratum conditions before being moved to storage.

The variables related to diaspore viability and vigor were verified to vary among the M. urundeuva mother trees. Consequently, it was possible to carry out principal component analysis with the scores, and an eigenvector circle may be used to differentiate between individuals and to identify the preponderant variables in this study. For the physiological quality of the diaspores, two components were necessary to explain satisfactorily the variability among mother trees. Cumulative variances of 86.7% and 92.6% were observed for principal components 1 and 2 (Table 4).

Table 4. Eigenvectors of two principal components of variables related to the physiological quality of *Myracrodruon urundeuva* diaspores collected in the municipalities of São João do Cariri and Boa Vista, state of Paraíba, Brazil.

Variables	Component 1	Component 2
Germination	0.394	0.342
First count of germination	0.388	0.460
Speed of germination index	0.417	0.442
Emergence	0.412	-0.410
First count of emergence	0.409	-0.399
Speed of emergence index	0.428	-0.385
Eigenvectors	4.690	0.782
Accumulated variances (%)	86.7	92,6

All the analyzed variables were important to the discrimination of groups in the principal component analysis. However, the germination and emergence tests ordered the mother trees differently in regard to the physiological quality of the diaspores. This differentiation was confirmed by the analysis (Figure 2), in which the germination and emergence tests are perfectly separated in axis 2 (Component 2), which explains 14.45% of the variance. However, the analysis revealed that component 1 explained 78.17% of the variance; this high value is an indication that more than one vigor test should be conducted in addition to the germination test.

Therefore, the continuous spatial distribution of the data was evidenced, and four clusters were defined according to the observed similarities in the physiological quality of diaspores (Figure 3). Among these, groups 1 and 2 were especially notable. Group 1 consisted of 15 mother trees in the first year (trees 1, 2, 3, 5, 7, 8, 12, 13, 15, 20, 22, 24, 25, 26, and 27), five in the second year (trees 7, 8, 16, 20, and 26) and one (tree 4) in the third year. Group 2 consisted of four mother trees in the first year only (trees 4, 9, 11, and 18).

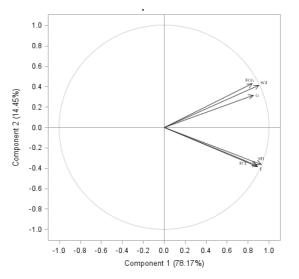


Figure 2. Eigenvector circle resulting from the principal components of the variables germination (G), first count of germination (FCG), speed of germination index (SGI), emergence (E), first count of emergence (FCE) and speed of emergence index (SEI) of *Myracrodruon urundeuva* diaspores collected during three consecutive years in the municipalities of São João do Cariri and Boa Vista in the state of Paraíba, Brazil.

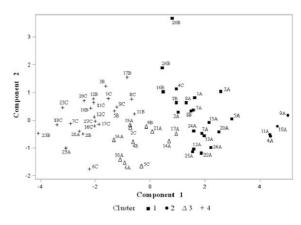


Figure 3. Scatterplot of *Myracrodruon urundeuva* diaspore samples, with four clusters based on two principal component scores (1 and 2) related to the physiological quality of diaspores. (Numbers indicate the mother trees; letters A, B, and C indicate years 1, 2, and 3 of collection, respectively.)

Groups 1 and 2 were clearly different from groups 3 and 4 owing to the predominant localization in the positive axis of component 1, where the highest-quality diaspores are found. It is necessary to emphasize that in the first year, the mother trees of group 2 produced diaspores of high physiological potential, scoring values above 4 for component 1.

As to group 1, trees 20 and 26, in the second year, reached high score values as expressed by component 2, representing the high potential of the diaspores of this group.

The two components explaining the variability between mother trees of this study differentiate important factors in seed quality evaluation: the controlled analyses under environmental conditions (germination test and related variables) and those simulating field conditions (seedling emergence test), in addition to the need for more than a vigor test in conjunction with the germination test (Carvalho & Nagakawa, 2012; Marcos Filho, 2015).

It was observed that some diaspores in groups 3 and 4 (the groups where the lowest-quality diaspores are found) were from the same mother trees as seeds in groups 1 and 2 (Figure 3) but were collected in different years. These results show that the collection period influenced the formation of clusters and that the origin (the area where the mother trees are located) had little influence on the data clustering, since mother trees from all three areas were found in the same groups.

According to the literature, seasonality is a preponderant factor in the physiological quality of seeds (Griz & Machado, 2001; Carvalho & Nakagawa, 2012; Marcos Filho, 2015, Nascimento et al., 2017). Nunes et al. (2008) say that the optimal moment for M. urundeuva seed harvest should be rigorously determined, since factors such as rainfall and temperature are determinants in the seed maturation process and, consequently, in the germination and seedling emergence process.

Year of collection had a substantial effect on diaspore physiological quality, as shown by the results of the three consecutive years of collection. Tree 4, for instance, may be considered of good quality since it is present in groups 1 and 2 in the third and second years of collection, even though factors occurring in the third year led to reductions in diaspore quality as evidenced by mother trees 7, 8, and 20, which were in group 1 in the first and second years of collection, but moved to group 4 in the third year (Figure 3).

Therefore, for the collection of M. urundeuva seeds, we recommend the mother trees forming group 1 because seeds from that group had the highest mean germination and emergence, at 74% and 67%, respectively, and the diaspores of some mother trees maintained high quality for more than one year of collection. Group 2 may also be considered adequate for the collection of seeds physiological potential high due to the (germination and emergence) shown by those seeds in the first year of collection (84% and 85%,

respectively), although this group was represented only by four mother trees.

Conclusion

The physiological quality of Myracrodruon urundeuva diaspores is dependent on environmental factors.

The physiological quality of M. urundeuva diaspores varies among mother trees, years and collection sites.

Trees in groups 1 and 2 are the most promising, since they contain representatives of the three areas and produce high physiological potential diaspores.

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