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Scientific Article

GENETIC DIVERSITY, MATING SYSTEM AND REPRODUCTIVE PHENOLOGY OF *Mimosa scabrella* BENTH: IMPLICATIONS FOR SEED COLLECTION

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ABSTRACT

Ecological restoration requires seed sources of native species with sufficient genetic diversity to maintain the evolutionary potential of subsequent populations. Studies elucidating genetic variability, mating system, and reproductive phenology are crucial for designing seed collection strategies. These aspects were evaluated in two populations of *Mimosa scabrella* (Bom Retiro-BR and Urupema-URU, Santa Catarina) to support such strategies. Genetic variation was analyzed using nine isozyme systems in 50 adult trees per population. The same markers were employed in open pollinated progeny arrays, from both populations, to evaluate the mating system. Phenological observations were conducted for one year in 30 individuals per population. Genetic variation revealed high diversity ($\hat{H}_E$ = 0.190 in BR; 0.245 in URU), but significant fixation indexes ($\hat{f}$ = 0.237 in BR; 0.156 in URU). The species exhibited a mixed mating system, predominantly outcrossed ($\hat{t}_m$ = 0.852 in BR; 0.801 in URU), with selfing, biparental inbreeding, and correlated matings, resulting in high coancestry and low effective variance size. To retain an effective size of 100 individuals non-inbred and unrelated, it is recommended to collect seeds from at least 37 seed trees in BR and 39 in URU, spaced at distances higher than 50 m. Phenological patterns showed synchronized winter flowering and fruit dehiscence between February and March, delayed in URU by one month, and influenced by climatic variables. These results confirm both populations as valuable seed sources for restoration, with specific recommendations to maximize genetic representativeness and adaptive potential.

Keywords: Bracatinga, Outcrossing rate, Gene flow

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DIVERSIDADE GENÉTICA, SISTEMA DE REPRODUÇÃO E FENOLOGIA REPRODUTIVA DE *Mimosa scabrella* BENTH: IMPLICAÇÕES PARA A COLETA DE SEMENTES

RESUMO A restauração ecológica requer fontes de sementes de espécies nativas com diversidade genética suficiente para manter o potencial evolutivo das populações subsequentes. Estudos que elucidem a variabilidade genética, o sistema de reprodução e a fenologia reprodutiva são cruciais para o delineamento de estratégias de coleta de sementes. Foram avaliados esses aspectos em duas populações de *Mimosa scabrella*. (Bom Retiro – BR e Urupema – URU, Santa Catarina) para subsidiar tais estratégias. A variação genética foi analisada utilizando nove sistemas isoenzimáticos em 50 árvores reprodutivas por população. Os mesmos marcadores foram empregados em progênies de polinização aberta, de ambas as populações, para avaliar o sistema de reprodução. Observações fenológicas foram conduzidas durante um ano em 30 indivíduos por população. A variação genética revelou alta diversidade ($\hat{H}_E$ = 0,190 em BR; 0,245 em URU), mas índices de fixação significativos ($\hat{f}$ = 0,237 em BR; 0,156 em URU). A espécie apresentou um sistema misto de reprodução, predominantemente alógamo ($\hat{t}_m$ = 0,852 em BR; 0,801 em URU), com autofecundação, endogamia biparental e cruzamentos correlacionados, resultando em elevada coancestria e baixo tamanho efetivo de variância dentro das famílias. Para manter um tamanho efetivo de 100 indivíduos não endogâmicos e não parentes, recomenda-se a coleta de sementes em pelo menos 37 matrizes em BR e 39 em URU, espaçadas em distâncias maiores do que 50 m. Os padrões fenológicos mostraram floração sincronizada no inverno e deiscência dos frutos entre fevereiro e março, atrasada em URU em um mês, e influenciada por variáveis climáticas. Esses resultados confirmam ambas as populações como fontes valiosas de sementes para restauração, com recomendações específicas para maximizar a representatividade genética e o potencial adaptativo.

Palavras-Chave: Bracatinga, Taxa de cruzamento, Fluxo gênico

1. INTRODUCTION

Intense deforestation driven by agricultural and urban expansion has reduced the Atlantic Forest to approximately 11% of its original cover, with most remnants consisting of secondary forest fragments smaller than 50 ha (Vancine et al., 2024). Fragmentation is a major cause of biodiversity loss, changes in ecological processes, and alterations in population genetic parameters (Santos et al., 2022). In this context, initiatives such as the Pact for the Restoration of the Atlantic Forest, which aims to restore 15 million hectares of the Atlantic Forest by 2050, have emerged to mitigate the impacts of deforestation and fragmentation (Schimetka et al., 2024).

However, the supply of native seeds does not meet the demand of growing restoration projects (Pedrini et al., 2020). To ensure long-term restoration success, it is essential to obtain quality propagules in sufficient quantity. Seed collection must follow criteria aimed at representing the genetic diversity necessary for population establishment in the short and long term (Prakash et al., 2024), such as a minimum number of seed trees and a minimum distance between them (Sebbenn, 2002; Iwaizumi et al., 2023). On the other hand, the high degree of fragmentation of the Atlantic Forest and the consequent loss of genetic diversity reduces the number of suitable sites for seed collection (Jalonen et al., 2018).

Therefore, it is important to characterize areas that can serve as sources of quality seeds, and one possible alternative is the establishment of Seed Collection Areas (SCAs).

According to Law No. 10,711/2003 and its Regulatory Decree (10.586/2020, Article 82), which established the National Cultivar Registry (RNC), SCAs can be defined as a population with selected seed trees, isolated from external pollen, with thinning of undesirable individuals and management for seed production, with informed individual selection criteria (Brasil, 2003).

The characterization of SCAs can be carried out considering a series of aspects,

such as genetic diversity, mating system, demography, and reproductive phenology. Estimates of neutral genetic diversity in forest fragment populations are useful for mapping regions with higher diversity indices, such as those carried out by the Forest SC Project (Reis et al., 2012; Montagna et al., 2018a). The mating system, in turn, can provide information regarding the effective variance size, a parameter used to estimate the number of seed trees required for seed collection in order to maintain a given effective population size (Sebbenn, 2002). Finally, demographic and phenological studies are fundamental to characterize populations with appropriate densities for seed collection, as well as the best times for collection and the associated variation (e.g., Mantovani et al., 2003; Montagna et al., 2018b; Rodrigues et al., 2023).

In addition to characterizing suitable areas for seed collection, the selection of species used in a restoration project is crucial for its success. *Mimosa scabrella* Benth. (Bracatinga) is a native tree of the Mixed Ombrophilous Forest, a forest typology within the Atlantic Forest. It is a fast-growing pioneer species, perennial, with a short life cycle and low demands in terms of soil edaphic conditions (Gerber et al., 2021). The species exhibits a mixed mating system with a predominance of outcrossing, which includes selfing, mating among relatives and correlated matings, resulting in open-pollinated families composed of mixtures of different relationships, such as self-sibs, half-sibs, and full-sibs (Sobierajski et al., 2006; Arruda et al., 2020). *Mimosa scabrella* is considered a facilitator species in the restoration process due to its relationship with nitrogen-fixing bacteria, insects and invertebrates, high biomass production, and adaptability to various soil edaphic conditions (Ferreira et al., 2019; Martins et al., 2020; Gerber et al., 2021). Furthermore, the species allows multiple uses - timber, honey production, ornamental, forage, fibers, dyes, gums and resins (Mazuchowski et al., 2014) and has been successfully used in restoration actions (Citadini-Zanette et al., 2017; Silva et al., 2019).

Given this scenario, this study aimed to characterize the genetic diversity, mating system, and reproductive phenology of two *Mimosa scabrella* populations used for seed collection in Santa Catarina, Brazil, in order to support local seed collection strategies for ecological restoration. Specifically, the study evaluated the genetic representativeness of the populations, the number of seed trees required to retain an effective population size of 100 individuals, and the most suitable seed collection period in each area.

2. MATERIAL AND METHODS

2.1 Study sites

The study was conducted in two forest fragments located in the municipalities of Urupema and Bom Retiro, in Santa Catarina state, Brazil (Figure 1). Both municipalities have a Köppen Cfb classification, indicating a temperate oceanic climate, with a total annual precipitation between 1,500 and 1,700 mm (Pandolfo et al., 2002). The remaining tree vegetation at both sites corresponds to Mixed Ombrophilous Forest. The studied forest fragments are located in Permanent Preservation Areas within two rural properties. Due to their heliophytic characteristic, the individuals were located mostly at the edges of the forest fragments, particularly in Urupema, where the population is located at a larger continuous fragment than in Bom Retiro.

2.2 Sampling and genetic analyses

To assess the neutral genetic diversity of each population in the studied fragments, 50 adult trees were tagged and georeferenced (Figure 1), and leaflets were collected for laboratory analyses. To reduce the likelihood of relatedness, a minimum distance of 50 m was maintained between sampled individuals. Nine isozyme markers were used to assess the levels of genetic diversity of the populations: Peroxidase (PRX), Phosphoglucomutase (PGM), Diaphorase (DIA), Phosphoglucoisomerase (PGI), Malic Enzyme (ME), Phosphogluconate (6PGDH), Isocitrate Dehydrogenase (IDH), Shikimate Dehydrogenase (SKDH), Beta-esterase (B-EST) (Alfenas, 1998).

The genetic diversity of adult individuals from the two populations was characterized using the following estimators: total number of alleles ($\hat{k}$), percentage of polymorphic loci ($\hat{P}$), mean number of alleles per locus ($\hat{A}$),

Revista ÁRVORE
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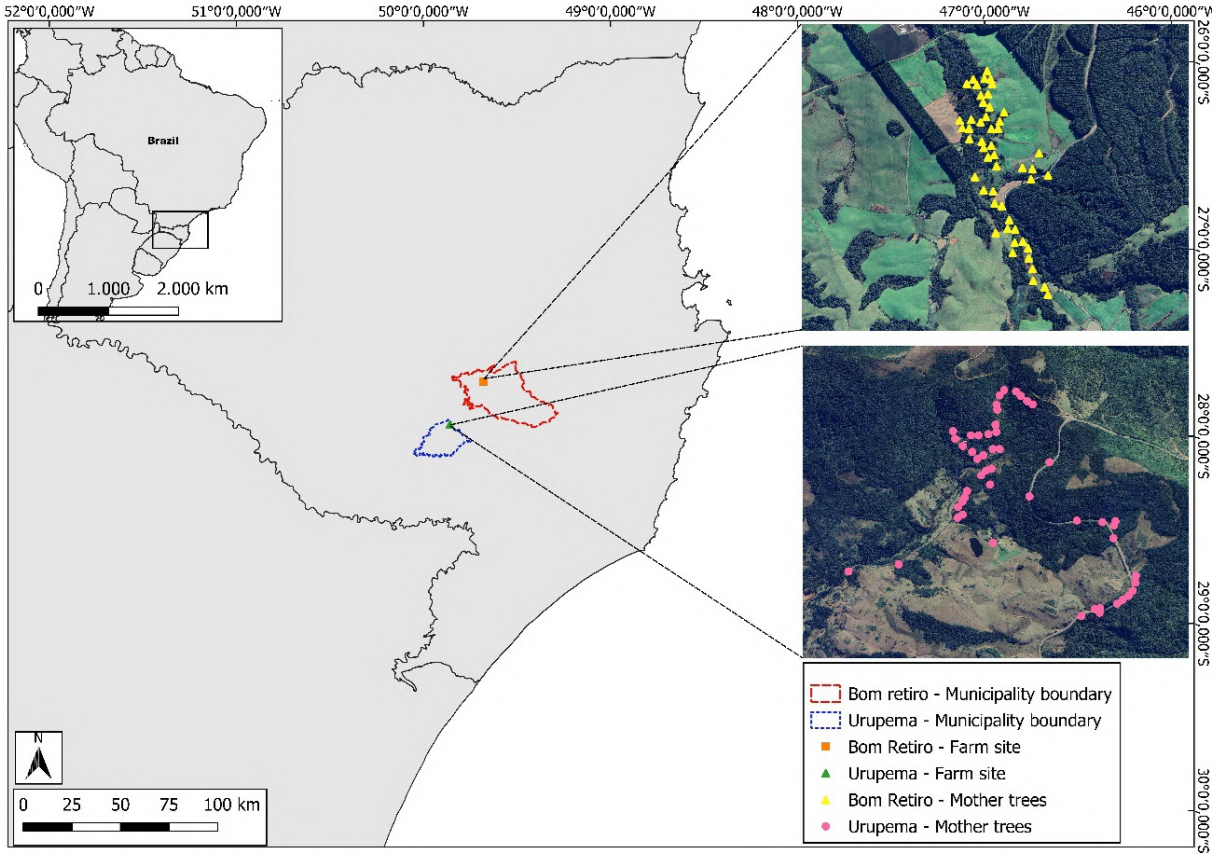


Figure 1. Map showing the location of *Mimosa scabrella* Benth.populations and individuals sampled in the municipalities of Bom Retiro and Urupema, Santa Catarina
Figura 1. Mapa mostrando a localização das populações de *Mimosa scabrella* Benth.e dos indivíduos amostrados nos municípios de Bom Retiro e Urupema, Santa Catarina

number of rare alleles (frequency < 5%), number of exclusive alleles, expected heterozygosity under Hardy-Weinberg equilibrium ($\hat{H}_E$), observed heterozygosity ($\hat{H}_0$), and fixation index ($\hat{f}$). The statistical significance ($p < 0.05$) of the $\hat{f}$ values was obtained through 1,000 permutations of alleles among individuals. All these analyses were performed using the FSTAT program, version 2.9.3.2 (Goudet, 2002).

To estimate parameters of the species mating system, approximately 100 fruits were collected from 12 and 13 seed trees in Bom Retiro and Urupema, respectively, between February (Bom Retiro) and March (Urupema) 2022, to form open-pollinated progeny arrays (hereinafter progeny: offspring from one seed tree). Sampling aimed to cover all crown strata of the seed trees, with fruits collected from two opposite cardinal points of the crown. Depending on field conditions and tree accessibility, collections were performed either along the North–South axis or along the East–West axis. After collection, 50 healthy seeds from each seed tree were selected (25 from each cardinal point), using only one seed per fruit. To overcome seed dormancy, seeds were immersed in water at 80 °C and left for 18 hours, with no additional heating. Subsequently, they were sown in tubes, which were placed in a greenhouse. When the seedlings reached approximately 15 cm in height, leaf samples from each individual were collected for genetic characterization using isozyme markers.

Twenty seedlings from each seed tree were genotyped. For the Bom Retiro progenies, seven loci were used (PRX1, PRX2, ME, DIA, PGI, 6PGDH, and SKDH), while for the Urupema progenies, eight loci were employed (PRX1, PRX2, ME, PGI, MDH - Malate Dehydrogenase, 6PGDH, IDH, and SKDH). Based on the assigned genotypes, the mating system was analyzed under the mixed-mating (Ritland & Jain,

1981) and correlated mating (Ritland, 1989) models, using the MLTR program, version 3.4 (Ritland, 2002). The following parameters were estimated: multilocus outcrossing rate ($\hat{t}_m$), single-locus outcrossing rate ($\hat{t}_s$), selfing rate ($\hat{s} = 1 - \hat{t}_m$), mating among relatives rate ($\hat{t}_m - \hat{t}_s$), selfing rate plus mating among relatives ($1 - \hat{t}_s$), multilocus paternity correlation ($\hat{r}_{p(m)}$), and inbreeding coefficient of seed trees ($\hat{F}_m$).

From the mentioned parameters, other parameters were estimated: effective number of pollen donors ($\hat{N}_{ep} = 1/\hat{r}_{p(m)}$) (Ritland, 1989), proportion of self-sibs ($\hat{P}_{ss} = \hat{s}$), half-sibs ($\hat{P}_{ss} = \hat{s}$), and full-sibs ($\hat{P}_{fs} = \hat{t}_m\hat{r}_{p(m)}$) (Sebbenn, 2002). The mean coancestry within progenies ($\hat{\theta}_{xy}$) was obtained by dividing by 2 the relatedness coefficient between plants within progenies $\hat{r}_{xy} = 0.250(1 + \hat{F}_m)[4\hat{s} + (\hat{t}_m^2 + \hat{t}_m\hat{s}\hat{r}_s)(1 + \hat{r}_{p(m)})]$ (Ritland, 1989), where $\hat{r}_s$ represents the selfing correlation. Based on $\hat{\theta}_{xy}$, the effective variance size was estimated ($\hat{N}_{e(v)} = 0.5/\hat{\theta}_{xy}$) (Cockerham 1969). Finally, the necessary number of seed trees to be sampled was estimated ($\hat{m} = N_{e(ref)}/\hat{N}_{e(v)}$) (Sebbenn, 2002), aiming to retain a reference effective size ($N_{e(ref)}$) of 100 unrelated and non-inbred individuals. Confidence intervals (95%) for each parameter were obtained through 1,000 bootstraps among progenies

2.3 Reproductive phenology

Phenological observations were carried out between July 2021 and March 2022, with 10-day intervals for initial phenophases and 30-day intervals for final phenophases, totaling 17 evaluations. These observations were made using binoculars on 30 plants randomly selected from the previously tagged seed trees in each population. The data obtained were used to determine the frequency of individuals in the following phenophases: 1) Flower buds; 2) Flowering; 3) Flower senescence; 4) Fruit formation; 5) Fruit maturation; 6) Fruit dehiscence (Rotta & Mendes, 1990). The phenological index for each phenophase was obtained by summing all intensity values different from zero divided by the number of individuals that showed phenological activity during a given month, modifying the proposal by Bencke & Morellato (2002).

Data analysis was performed using a dendrophenogram, which is a temporal representation of phenophase activity and their intensities (Fournier, 1976). The intensity of each phenophase was quantified using the scale proposed by Fournier (1974). Spearman correlation coefficients were also estimated to test the association of meteorological data (mean, maximum, and minimum temperatures, relative humidity and precipitation) with the frequency of occurrence of phenophases. The statistical significance of these correlations was tested in RStudio software using the cor.test function with the "spearman" method (R Development Core Team 2023). This procedure performs a hypothesis test to verify if the observed correlation coefficient is significantly different from zero, using an approximate test based on Student's t-distribution with n - 2 degrees of freedom, where n is the sample size. Meteorological data were obtained from Epagri/Ciram, from stations located in Urupema (code 1064) and Bom Retiro (code 2423).

3. RESULTS

3.1 Genetic diversity

Ten polymorphic loci were obtained with the nine isozyme systems used. The total number of alleles found was 31 in BR and 34 in URU, and the number of alleles per locus was 3.1 and 3.4, respectively (Table 1). Locus polymorphism was 100% at both sites.

The expected and observed heterozygosity values were 0.190 and 0.145 in BR and 0.254 and 0.208 in URU, resulting in positive and significantly higher than zero fixation indices in BR (0.237) and URU (0.156). More rare alleles were found in BR (15) compared to URU (11). Regarding exclusive alleles, URU presented five, while BR presented two.

3.2 Mating system

Both populations showed $\hat{t}_m$ different from unity, resulting in significant selfing rates (Table 2). A substantial proportion of matings were correlated ($\hat{r}_{p(m)}$ = 0.510 and 0.395 for BR and URU, respectively). Selfing, correlated crosses, and mating among relatives resulted in progenies composed of self-sibs ($\hat{P}_{ss}$ = 0.148 and 0.199 for BR and URU, respectively), half-sibs ($\hat{P}_{hs}$ = 0.621 and 0.485 for BR and URU, respectively), and full-sibs ($\hat{P}_{fs}$ = 0.231 and

Table 1. Estimates of genetic diversity indices in *Mimosa scabrella* Benth. populations in two forest fragments in Santa Catarina (Bom Retiro and Urupema)
Tabela 1. Estimativas de índices de diversidade genética em populações de *Mimosa scabrella* Benth. em dois fragmentos florestais em Santa Catarina (Bom Retiro e Urupema)

	Bom Retiro	Urupema
Sample size (n)	48	48
Total number of alleles ($\hat{K}$)	31	34
Percentage of polymorphic loci ($\hat{P}$)	100	100
Mean number of alleles per locus ($\hat{A}$)	3.1	3.1
Expected heterozygosity ($\widehat{H}_E$)	0.190	0.245
Observed heterozygosity ($\widehat{H}_O$)	0.145	0.208
Fixation index ($\hat{f}$)	0.237*	0.156*
Number of rare alleles (Rares)	15	11
Number of private alleles (Private)	2	5

*P< 0,05.

0.316 for BR and URU, respectively), and progeny arrays of both populations with coefficient of coancestry higher than expected for half-sib families ($\hat{\theta}_{xy}$ = 0.125), resulting in effective variance sizes of 2.760 for BR and 2.644 for URU. Thus, aiming to retain an effective population size corresponding to 100 unrelated and non-inbred individuals, it is necessary to collect seeds from 36.2 seed trees in BR and 37.8 seed trees in URU.

3.3 Reproductive phenology

The results of the phenological evaluation are shown in Figures 2 (BR) and 3 (URU). The assessments allowed characterization of one reproductive cycle in each population, albeit incompletely for the

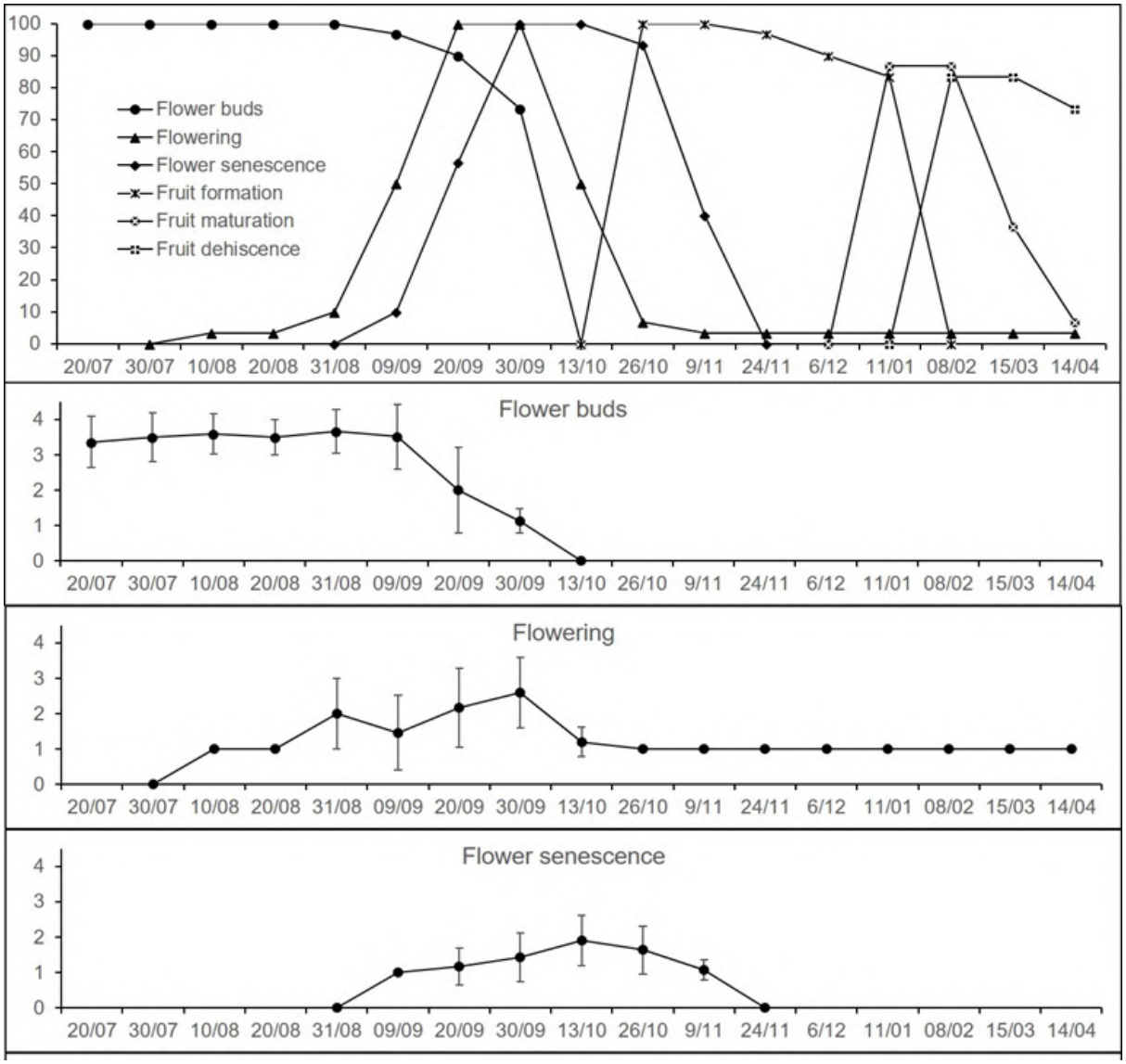
Table 2. Mating system in *Mimosa scabrella* Benth. populations in two forest fragments, Bom Retiro and Urupema, Santa Catarina
Tabela 2. Sistema de reprodução em populações de *Mimosa scabrella* Benth. em dois fragmentos florestais, em Bom Retiro e Urupema, Santa Catarina

	Bom Retiro (7 loci)	Urupema (8 loci)
Seed trees (progenies)	12 (280)	13 (240)
Multilocus outcrossing rate ($\hat{t}_m$)	0.852 (0.762/0.970)	0.801 (0.650/0.960)
Single-locus outcrossing rate ($\hat{t}_s$)	0.739 (0.611/0.916)	0.718 (0.545/0.891)
Multilocus paternity correlation ($\hat{r}_{p(m)}$)	0.510 (0.192/0.637)	0.395 (0.153/0.559)
Mating among relatives ($\hat{t}_m - \hat{t}_s$)	0.113 (0.032/0.166)	0.083 (0.029/0.148)
Selfing rate ($\hat{s}$)	0.148 (0.030/0.238)	0.199 (0.040/0.350)
Selfing plus mating among relatives ($1 - \hat{t}_s$)	0.261 (0.084/0.389)	0.282 (0.109/0.455)
Proportion of self-sibs ($\hat{P}_{ss}$)	0.148 (0.030/0.238)	0.199 (0.040/0.350)
Proportion of half-sibs ($\hat{P}_{hs}$)	0.621 (0.520/0.749)	0.485 (0.296/0.792)
Proportion of full-sibs ($\hat{P}_{fs}$)	0.231 (0.152/0.328)	0.316 (0.132/0.381)
Effective number of pollen donors ($\widehat{N}_{ep}$)	3.7 (2.7/5.6)	2.5 (1.8/4.3)
Coefficient of relatedness within progenies ($\hat{r}_{xy}$)	0.362 (0.268/0.453)	0.378 (0.261/0.479)
Coefficient of coancestry within progenies ($\hat{\theta}_{xy}$)	0.181 (0.134/0.227)	0.189 (0.131/0.239)
Effective variance size ($\widehat{N}_{e(v)}$)	2.760 (2.207/3.738)	2.644 (2.088/3.825)
Number of seed trees for seed collection ($\widehat{m}_{100}$)	36.2 (26.6/45.3)	37.8 (47.9/26.1)

The number of seed trees for seed collection ($\widehat{m}_{100}$) was estimated aiming to retain a reference effective size of 100 individuals; between parenthesis: 95% confidence intervals.
O número de matrizes para coleta de sementes ($\widehat{m}_{100}$) foi estimado visando reter um tamanho efetivo de referência de 100 indivíduos; entre parênteses: intervalos de confiança de 95%.

flower bud and fruit dehiscence phenophases. Both populations had 100% of individuals with flower buds in July, with flowering starting in July/21 in BR and August/21 in URU. The BR population showed 100% of individuals flowering during September/21, while in URU the maximum percentage of flowering individuals reached 96.6% in the October/21 assessment. Both populations showed fruit formation starting in October/21 and fruit maturation in January/22, maintaining ripe fruits until the May/21 assessment. Fruit dehiscence, in turn, began in February/22 for both populations. Although both areas are located in the same geographic region of SC, there was variation between them. In general, the phenological cycle of URU was one month later compared to BR. The intensities of each phenophase were decreasing, in both populations, from flower buds to fruit dehiscence, indicating a greater quantity of buds and flowers than the resulting quantity of fruits.

There is evidence of climatic influence on the reproductive phenology of *M. scabrella* in both populations. All correlations between climatic variables and phenophases are shown in Table 3. The flower bud phenophase was negatively correlated, in



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Revista ÁRVORE
ISSN 1806 - 9088

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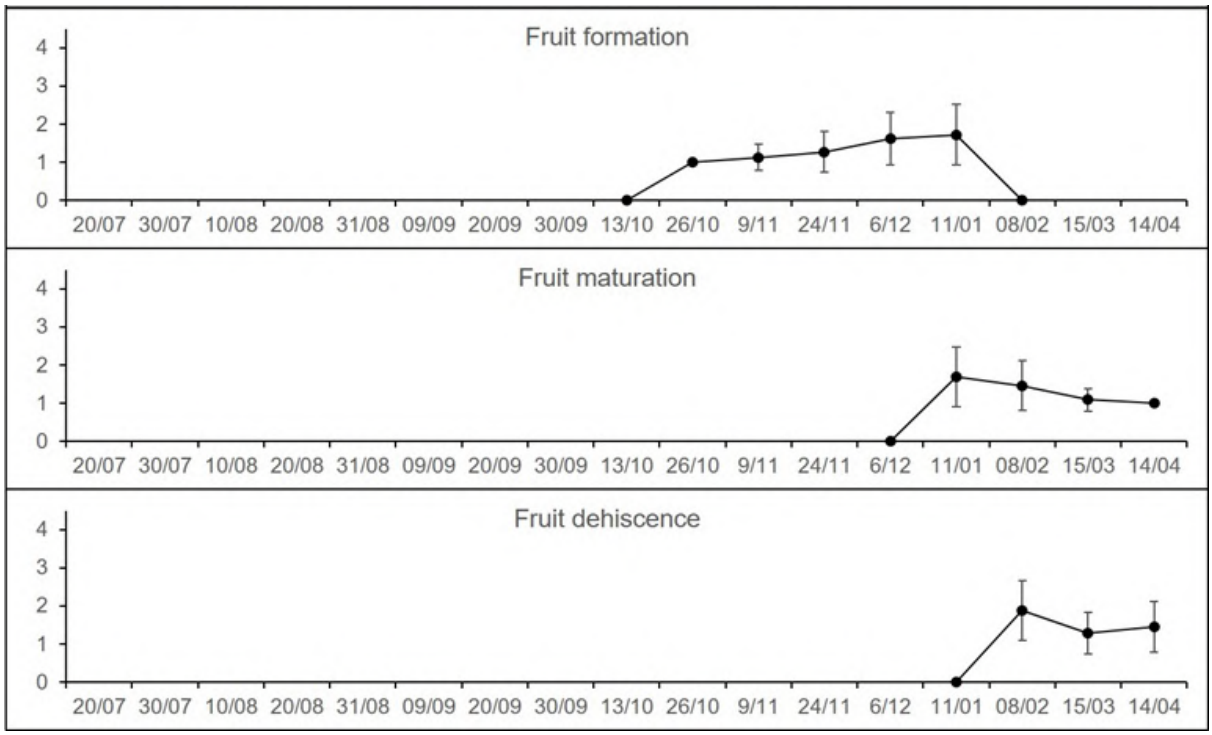
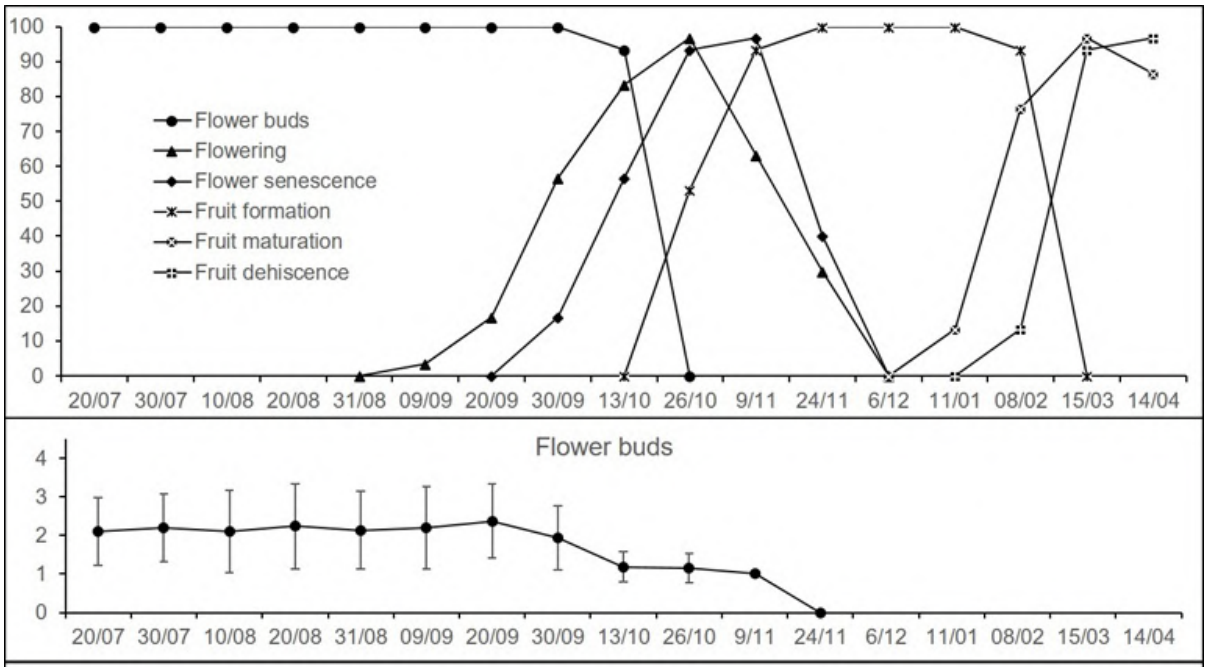


Figure 2. Reproductive phenology of *Mimosa scabrella* Benth. in Bom Retiro (SC). From top to bottom: percentage of individuals presenting a given phenophase; phenological index for each evaluated phenophase: flower buds, flowers, senescing flowers, fruit formation, fruit maturation, and fruit dehiscence. Evaluations occurred between July 2021 and April 2022. Error bars represent the standard deviation for intensity in each observation

Figura 2. Fenologia reprodutiva de *Mimosa scabrella* Benth. em Bom Retiro (SC). De cima para baixo: porcentagem de indivíduos apresentando uma determinada fenofase; índice fenológico para cada fenofase avaliada: botões florais, flores, flores em senescência, formação de frutos, maturação de frutos e deiscência de frutos. As avaliações ocorreram entre julho de 2021 e abril de 2022. As barras de erro representam o desvio-padrão da intensidade em cada observação



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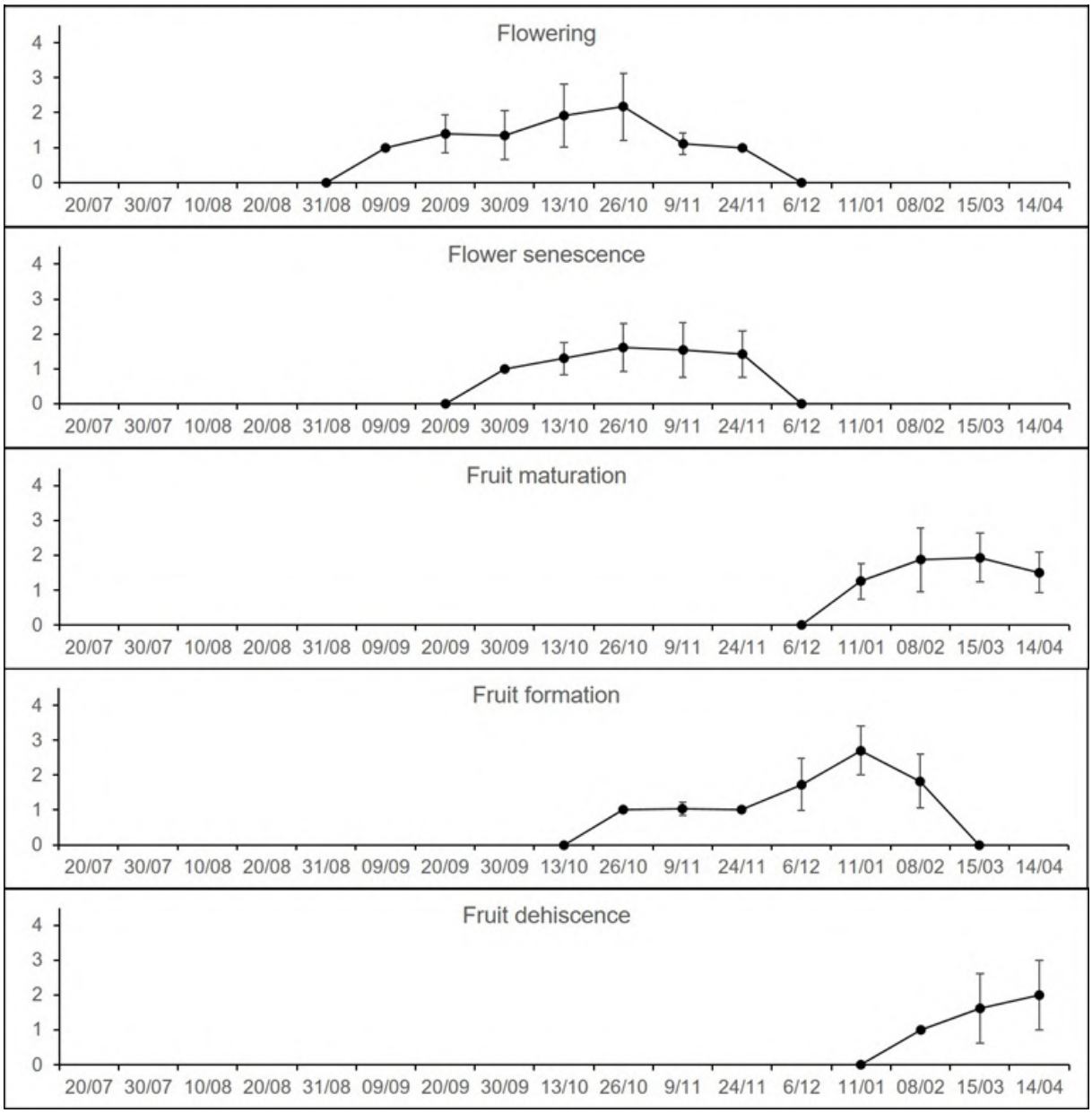

Figure 3. Reproductive phenology of *Mimosa scabrella* Benth. in Urupema (SC). From top to bottom: percentage of individuals presenting a given phenophase; phenological index for each evaluated phenophase: flower buds, flowers, senescing flowers, fruit formation, fruit maturation, and fruit dehiscence. Evaluations occurred between July 2021 and April 2022. Error bars represent the standard deviation for intensity in each observation

Figura 3. Fenologia reprodutiva de *Mimosa scabrella* Benth. em Urupema (SC). De cima para baixo: porcentagem de indivíduos apresentando uma determinada fenofase; índice fenológico para cada fenofase avaliada: botões florais, flores, flores em senescência, formação de frutos, maturação de frutos e deiscência de frutos. As avaliações ocorreram entre julho de 2021 e abril de 2022. As barras de erro representam o desvio-padrão da intensidade em cada observação

both populations, with maximum, mean, and minimum temperatures, suggesting that drops in temperature are related to the beginning of the reproductive cycle. For flowering, a positive correlation with relative humidity was found for both locations. The fruit maturation and dehiscence phenophases were positively correlated with maximum, mean, and minimum temperatures in both populations. Finally, some correlations were specific to each population, such as the positive correlation between precipitation and

Revista ÁRVORE
ISSN 1806 - 9088

flower senescence in BR; and the positive correlations between relative humidity and maximum temperature and flower senescence and fruit formation, respectively, in URU.

4. DISCUSSION

4.1 Genetic diversity

Both *M. scabrella* populations showed high genetic variability for adult individuals,

Table 3. Spearman correlation between climatic variables and phenophases of *Mimosa scabrella* Benth. in the municipalities of Bom Retiro and Urupema, Santa Catarina
Tabela 3. Correlação de Spearman entre variáveis climáticas e fenofases de *Mimosa scabrella* Benth. nos municípios de Bom Retiro e Urupema, Santa Catarina

	TMax	TMed	TMin	UR	P
Bom Retiro					
BF	-0.85*	-0.90*	-0.86*	0.35	-0.35
FL	-0.39	-0.36	-0.37	0.63*	0.46
SEN	-0.12	0.01	0.06	0.41	0.71*
FF	0.42	0.42	0.46	-0.36	0.02
MF	0.67*	0.67*	0.58*	-0.37	-0.10
FD	0.55*	0.55*	0.52*	-0.15	0.08
Urupema					
BF	-0.72*	-0.75*	-0.80*	-0.43	-0.36
FL	-0.12	-0.18	-0.13	0.55*	0.47
SEN	-0.12	-0.17	-0.09	0.60*	0.22
FF	0.51*	0.48	0.46	0.13	0.13
MF	0.58*	0.61*	0.65*	0.17	0.20
FD	0.44*	0.48*	0.55*	0.29	0.24

BF = Flower buds; FL = Flowering; SEN = Flower senescence; FF = Fruit formation; MF = Fruit maturation; FD = Dehiscent fruits; TMax = Maximum temperature; TMed = Mean temperature; TMin = Minimum temperature; UR = Relative Humidity; P = Precipitation. *P< 0.05.
BF = Botões florais; FL = Floração; SEN = Senescência floral; FF = Formação de frutos; MF = Maturação de frutos; FD = Frutos deiscentes; TMax = Temperatura máxima; TMed = Temperatura média; TMin = Temperatura mínima; UR = Umidade relativa; P = Precipitação. *P< 0,05.

a result consistent with previous studies of the species in Southern Brazil (Sobierajski et al., 2006; Moreira et al., 2011; Arruda et al., 2019) and with the general trend in tree species (Loveless & Hamrick, 1984; Sebbenn, 2002). The presence of rare and private alleles, also reported by Moreira et al. (2011) and Arruda et al. (2019) highlights the potential of both populations to retain unique genetic variations for adaptation to environmental changes.

Despite the high levels of diversity, a deficit of heterozygotes was observed in both populations, resulting in positive fixation indices, a common pattern in species subject events such as inbreeding and spatial genetic structuring (Hedrick, 2004). This excess of homozygotes results, in part, from selfing and mating among relatives, as estimated for seedlings of the populations (Table 2). However, it may also result from genetic drift in small or fragmented populations, as previously reported for *M. scabrella* (Sobierajski et al., 2006; Moreira et al., 2011).

The high genetic diversity of the species can be partly explained by its predominantly allogamous mating system, favored by abundant pollen release (Carpanezzi & Laurent, 1988; Vergamini et al., 2006). Although seed dispersal occurs through autochory, long-distance gene flow can be maintained by pollinators such as Apis mellifera, especially at low densities (Dick et al., 2008; Moreira et al., 2011). Land-use history may also influence the high genetic diversity levels, as disturbances and traditional agroforestry management may have promoted generational overlap and accumulation of diversity in the seed bank (Moreira et al., 2011).

4.2 Mating system

Mimosa scabrella exhibits a mixed mating system, predominantly allogamous, but with selfing and mating among relatives. Multilocus outcrossing rates, lower than 1, confirm moderate levels of selfing, consistent with previous estimates for the species (Sobierajski et al., 2006; Moreira et al., 2011; Arruda et al., 2020). This pattern characterizes mixed systems, which combine advantages of allogamy and selfing (Goodwillie et al., 2005; Whitehead et al., 2018).

The difference between multilocus and single-locus outcrossing rates indicates biparental inbreeding, as also reported by Sobierajski et al. (2006) and Arruda et al. (2020). In aggregated populations, spatial proximity favors mating among neighbors, even with pollinators capable of long flights, since floral fidelity and sequential behavior lead to local visits (Zurbuchen et al., 2010). This effect, combined with autochorous seed dispersal, maintains spatial genetic structure, a pattern also described in *Anadenanthera colubrina* (Feres et al., 2021) and *Copaifera langsdorffii* (Manoel et al., 2012).

Estimates of paternity correlation and within-progeny coancestry confirm that families are not composed only of half-sibs, but also of full-sib and self-sib individuals, as observed in previous studies (Sobierajski et al., 2006; Arruda et al., 2020). This pattern may result from flowering synchrony and the large number of flowers per individual, which favor sequential visits by pollinators to neighboring trees (Devaux et al., 2014).

The effective variance size (2.760 in BR and 2.644 in URU) showed values compatible with those estimated in other populations of the species (Sobierajski et al., 2006; Arruda et al., 2019). The substantial proportion of self-sibs and full-sibs in the progenies reduces the genetic contribution of each seed tree for seed lots, requiring a larger number of trees to ensure representativeness. Thus, to retain an effective size of 100 individuals, at least 37 seed trees in BR and 39 in URU would be required, above the 30 generally recommended for fully allogamous species (Brown & Hardner, 2000).

4.3 Reproductive phenology

Mimosa scabrella presented an annual cycle, with flower buds appearing in July and fruit dehiscence starting in February. However, there were differences between populations, as phenophases in URU started and ceased one month later than in BR, possibly due to altitude and climate effects (Deng et al., 2022; Jiang et al., 2023), since BR has higher temperatures than URU. Phenophases showed decreasing intensities, with high flowering synchrony, a pattern already described for the species (Carpanezzi & Laurent, 1988; Rotta & Mendes, 1990). A greater production of flowers compared to fruits was also observed, indicating limitations in the conversion to viable seeds (Rotta & Mendes, 1990).

Climatic variables influenced phenology. The negative correlation between flower buds and temperature suggests that cooling triggers flowering, as in other species that bloom in winter (Bencke & Morellato, 2002). In contrast, fruit maturation and dehiscence showed positive correlation with temperature, indicating that warming favors development and release, a common pattern in species that complete their cycle between summer and autumn (Bergamaschi, 2007; Bencke & Morellato, 2002). Specific differences, such as the association between precipitation and senescence in BR or between humidity, temperature and fruiting in URU, suggest influences of microclimate, altitude and topography on the duration and intensity of phenophases.

Dehiscence was concentrated between February and March, indicating that this period is the most suitable for collecting viable seeds in the studied populations. The phenological difference of up to one month between populations highlights the importance of local monitoring, even in nearby areas, due to microclimatic variations (Bergamaschi, 2007; Costa et al., 2022). Understanding reproductive phenology is essential for species used in restoration, as it guides seed collection (Buisson et al., 2017; Costa et al., 2023), allows evaluation of event synchronization (Solga et al., 2014), and helps comprehend responses to local climate variations (Borchert et al., 2004; Morellato et al., 2016).

Revista ÁRVORE
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4.4 Recommendations for seed collection

To maximize genetic representativeness in restoration programs, seed collection from at least 37 seed trees in BR and 39 in URU is recommended. If sampling can be spread across different reproductive events, there is a high probability to increase the effective number of parents. Seed collection should be concentrated between February and March, when most viable fruits are released. A general recommendation is to collect similar quantities of seeds from each seed tree, thus preventing a reduction in effective population size caused by unequal parental contributions. Another general recommendation is to ensure that seed trees are spaced (~50 m) to minimize sampling from related individuals.

5. CONCLUSION

This study demonstrates that the *M. scabrella* populations from BR and URU possess attributes that qualify them as seed sources for restoration, but with relevant differences that should guide collection strategies. Both show high genetic diversity and exclusive alleles, essential for conserving the species' adaptive potential. However, the heterozygote deficit and high coancestry reveal the need to increase the number of sampled seed trees, with minimum estimates of 36 trees in BR and 38 in URU. The reproductive phenology, characterized by high flowering synchrony and concentrated fruit dehiscence between February and March, defines a strategic period for collection, which should be distributed spatially and temporally to reduce genetic redundancy. The results obtained provide relevant information for seed collection planning in the studied populations, including recommendations regarding sampling intensity and collection period.

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AUTHOR CONTRIBUTIONS

Scussel, G. F.: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing; Rodrigues, I. C. A.: Investigation, Methodology, Writing – review & editing; Farias, A. C. A.: Methodology, Writing – review & editing; Ferreira, J. M.: Investigation, Methodology, Writing – review & editing; Machado, F. I.: Investigation, Methodology, Writing – review & editing; Montagna, T.: Conceptualization, Formal analysis, Validation, Writing – review & editing; Reis, M. S.: Conceptualization, Funding acquisition, Validation, Writing – review & editing.

DATAAVAILABILITY

The entire dataset supporting the findings of this study has been published within the article.

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