

REPRODUCTIVE SYSTEM of *Mimosa scabrella* IN CONSECUTIVE REPRODUCTIVE EVENTS IN THE MOUNTAINOUS REGION OF SANTA CATARINA STATE

Guilherme Oliveira Santos Ferraz de Arruda^{1*}, Adelar Mantovani², Tiago Montagna⁴, Alison Paulo Bernardi⁴, Newton Clóvis Freitas da Costa³, Maurício Sedrez dos Reis⁴

^{1*}State University of Santa Catarina, Center for Agroveterinary Sciences, Postgraduate Program in Plant Production, Lages, Santa Catarina, Brazil - * guilherme.fdearruda@gmail.com

²State University of Santa Catarina, Center for Agroveterinary Sciences, Postgraduate Program in Plant Production, Research Group in Use and Conservation of Forest Resources, Lages, Santa Catarina, Brazil - adelar.mantovani@udesc.br

³State University of Santa Catarina, Center for Agroveterinary Sciences, Laboratory of Genetic Analysis, Lages, Santa Catarina, Brazil - newtoncfc@gmail.com

⁴Federal University of Santa Catarina, Nucleus for Research in Tropical Forests, Postgraduate Program in Plant Genetic Resources, Florianópolis, Santa Catarina, Brazil - montagna@gmail.com, bernardialison@gmail.com, msedrez@gmail.com

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Resumo

Sistema reprodutivo de Mimosa scabrella em eventos reprodutivos consecutivos no Planalto Serrano Catarinense. O sistema reprodutivo determina a herança evolutiva de populações ao estabelecer padrões de união dos gametas para formar a geração seguinte. O objetivo deste estudo foi investigar o sistema reprodutivo de *Mimosa scabrella* Benth. (bracatinga), espécie arbórea brasileira com importância socioeconômica e ecológica, em população do planalto serrano do estado de Santa Catarina, em dois eventos reprodutivos consecutivos. As análises dos eventos reprodutivos de 2015 e 2016 foram conduzidas com marcadores isoenzimáticos e a caracterização do sistema reprodutivo foi realizada com base nos modelos de cruzamentos mistos e cruzamentos correlacionados, sendo as médias de cada parâmetro comparadas estatisticamente. Dentre os resultados, as taxas de cruzamento multiloco ($t_{m(2015)}$: 0,925 e $t_{m(2016)}$: 0,845) apresentaram magnitude alta e diferiram da unidade; as taxas de autofecundação foram significativamente diferente de zero ($s_{(2015)}$: (0,075 e $s_{(2016)}$: 0,155), indicando que a espécie não apresenta autoincompatibilidade. Além disso, as taxas positivas de cruzamentos entre indivíduos aparentados ($t_{m-t_s(2015)}$: 0,142 e $t_{m-t_s(2016)}$: 0,170) sugerem a ocorrência de cruzamentos endogâmicos na população. Em relação aos descendentes, as maiores proporções foram de meio-irmãos (PMI $_{(2015)}$: 0,736 e PMI $_{(2016)}$: 0,579), sugerindo o predomínio da aleatoriedade nos cruzamentos ocorridos. Conclui-se que a espécie *M. scabrella* possui sistema reprodutivo misto, com predominância de cruzamentos na população.

Palavras-chave: Bracatinga, Cruzamentos, Autofecundação, Sistema Misto. .

Abstract

The reproductive system determines evolutionary inheritance of populations by establishing patterns of gametes union to originate the next generation. The objective of this study was to investigate the mating system of *Mimosa scabrella* Benth. (bracatinga), a Brazilian tree species with socioeconomic and ecological importance, in a population of the mountainous region of Santa Catarina state, in two consecutive reproductive events. The analyzes in the 2015 and 2016 reproductive events were lead with isoenzymatic markers and the mating system characterization was performed based on mixed crosses and correlated crosses models, and the means of each parameter were statistically compared. Among the results, the multilocus outcrossing rates ($t_{m(2015)}$: 0.925 and $t_{m(2016)}$: 0.845) showed high magnitude and differed from the unit; the self-fertilization rates were significantly different from zero ($s_{(2015)}$: (0.075 and $s_{(2016)}$: 0.155) indicating that the species does not show self-incompatibility. In addition, the positive outcrossing rates among relatives ($t_{m-t_s(2015)}$: 0.142 and $t_{m-t_s(2016)}$: 0.170) suggest inbreeding crosses in the population. The highest proportions in offspring were half-sibs ($_{(2015)}$: 0.736 and $_{(2016)}$: 0.579), suggesting predominance of randomness at crossings. From this it is concluded that *M. scabrella* has a mixed mating system, with predominance of crossing at population level.

Keywords: Bracatinga, crossings, self-fertilization, mixed system.

INTRODUCTION

In tree species, the study of the reproductive system is a primary step to find out estimates of crossbreeding in a population and to determine the mode of transmission of genes to descendants (MORI *et al.*, 2013). For species of socioeconomic and ecological interest, knowing the reproductive system helps with information for conservation programs, genetic improvement and quantification of the number of trees for the collection of seeds

in recovering plantations (MORI *et al.*, 2013). In tree species, reproduction can occur through crossings (random and biparental), self-fertilization and combinations of these, in addition to apomixis, modalities that can be elucidated with the help of genetic markers (GUSSON *et al.*, 2006).

Mimosa scabrella Benth. (Fabaceae) or bracinga, is a pioneer tree, occurring in the Mixed Ombrophilous Forest. It reaches reproductive age at age three, but it can bloom earlier in exceptional environmental conditions (CARPANEZZI *et al.*, 1997). Its inflorescences are of the flower head type, with male flowers and protogenic hermaphrodites presenting the *mass-flowering* phenomenon, with bees the most effective pollinators, such as those of the genera *Trigona*, *Melipona* and *Plebeia*, in addition to the Africanized *Apis mellifera* (HARTER-MARQUES; ENGELS, 2003). The seeds disperse by autochory, naturally detaching from craspids and forming a seed bank in the soil, close to the matrix. The species' longevity is low, ending the cycle around 25 years (SIMINSKI; MAZUCHOWSKI, 2014).

Considered a kind of multiple use, it offers both wood and non-wood products, which gives it socioeconomic and ecological importance, especially in the areas of occurrence. Its wood is easy to work with when machining, giving it aptitude for furniture making. It is also used for floors, panels, boxwork, props and a source of heat energy, such as firewood or coal (MAZUCHOWSKI *et al.*, 2014).

For non-timber purposes, leaf biomass serves as a complementary feed to cattle in periods of scarce pasture. *M. scabrella* is known to be an outstanding bee species, as it is an important source of nectar and pollen during flowering in winter, when the supply of food resources to pollinators is incipient. In addition to floral nectar and pollen, there are sweet exudates ("melato") from living parts of the plant, expelled by scale insects, mainly from the genera *Tachardiella* and *Stigmacoccus* (WOLF *et al.*, 2015). Collected by honey bees, exudates originate honey with different physical-chemical characteristics from floral honey, rich in sugars and antioxidants (SERAGLIO *et al.*, 2016).

Under the ecological focus, the species is a successional facilitator and acts as a supplier of organic matter to the soil by providing its biomass in the leaf litter (FERREIRA *et al.*, 2016). It also contributes as a soil recuperator, fixing atmospheric nitrogen in symbiotic association with groups of bacteria, such as strains of the genus *Rhizobium* and *Burkholderia nodosa* (PRIMIERI *et al.*, 2016). By sequestering carbon from the atmosphere and stocking it in its biomass, the species has excellent potential for use in projects aimed at reducing greenhouse gases, in addition to compensations such as carbon credit (FERREIRA *et al.*, 2016).

The hypothesis elaborated for this study was that the reproductive system of *M. scabrella* is not altered after sequential reproduction events. The objective of the study was to investigate the reproductive system of *M. scabrella* during two consecutive reproductive events in an existing population in the municipality of Lages, at mountainous region of Santa Catarina state.

MATERIAL AND METHODS

Place of study and sampling

The study was carried out at the Experimental Farm of the Center for Agroveterinary Sciences (FECAV), belonging to State University of Santa Catarina (UDESC), in the municipality of Lages (Figure 1).

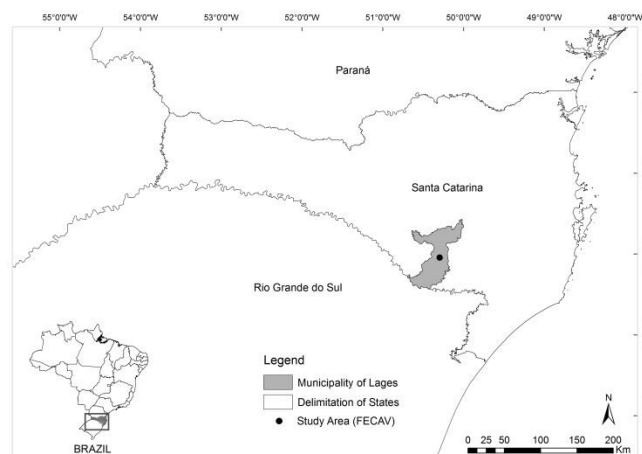


Figure 1. Geographical position of the municipality of Lages in the state of Santa Catarina.
Figura 1. Posição geográfica do município de Lages no estado de Santa Catarina.

The site has a total area of 191 ha, is located at latitude 27°45'S, longitude 50°04'W and altitude of 895 m. The climate is Cfb by the Köppen classification, with annual averages of 16.6 °C and 1441 mm of precipitation (CLIMATE, 2018) and the tree vegetation is remnants of Mixed Ombrophilous Forest. The isoenzymatic analyses were carried out at the Laboratory of Developmental Physiology and Plant Genetics of the Center of Agrarian Sciences, belonging to Federal University of Santa Catarina (UFSC), in Florianópolis-SC.

In the reproductive period of 2015, matrix trees of *M. scabrella* were chosen for population sampling, whose criteria were: having reproductive age, good phytosanitary status, having no evidence of senescence and being at least 50 m apart. From these matrices, fruits were collected and seeds extracted to produce progenies and compose the "families" (matrix and progenies) for analysis. A minimum of 15 seedlings per matrix was established to validate a "family" as a sample and, by this criterion, leaves from 23 matrices and 345 progenies were collected as fresh biological material for isoenzymatic analyses.

In the reproductive period of 2016, with the same 23 matrices analyzed in the previous year, the steps mentioned for the purposes of analysis related to this consecutive reproductive event were repeated.

Exsiccates of matrices of *M. scabrella* of the study population were incorporated into the collection of the Herbarium Lages of the State University of Santa Catarina (LUSC), located in Lages-SC, with registration LUSC 8327 (barcode LUSC 003743) and LUSC 8650 (barcode LUSC 003767).

Isoenzymatic analysis

Isoenzymatic markers were used to characterize the reproductive system of *M. scabrella*, since they are suitable for this purpose (CONTE *et al.*, 2008). For extraction and solubilization of leaf tissue isoenzymes, a macerator/homogenizer equipment of samples was used in rotation of 6,800 rpm for 15 seconds and extraction buffer solution number 1 (ALFENAS, 1998). Wicks (Whatman paper number 3, with 2 mm x 20 mm) were soaked in the solubilized extracts of the samples and inserted into the starch gel plate (Penetrose™ 30 to 13 %) migration of isoenzymes by electric current. Electrophoresis occurred in a refrigerator (5°C), with a buffer solution of the Tris-Citrate (TC) gel/electrode system pH 7.5. Nine isoenzymatic systems were used for the genetic characterization of the matrices and progenies: phosphogluco isomerase (PGI; EC 5.3.1.9), 6-phosphogluconate dehydrogenase (6PGDH; EC 1.1.1.44), phosphoglucomutase (PGM; EC 5.4.2.2), shikimate dehydrogenase (SKDH; EC 1.1.1.25), isocitrate dehydrogenase (HDI; EC 1.1.1.42), peroxidase (PRX; EC 1.11.1.7), malic enzyme (ME; 1.1.1.40), beta esterase (β -EST; EC 3.1.1.1) and diaphorase (DIA; EC 1.8.1.4).

Reproductive system analysis

To characterize the reproductive system of the species, in the two consecutive reproductive events, the Multilocus Mating System Program (MLTR - version 3.2) (RITLAND, 2008) was used. The means of the parameters were estimated by the maximum likelihood method (MLE) and compared to each other for both reproductive events by the Confidence Intervals (CI) with 95% confidence level. The parameters estimated for reproductive system were: multilocus outcrossing rate (t_m); single locus outcrossing rate (t_s); outcrossing rate among relatives ($t_m - t_s$); self-fertilization rate (s); self-fertilization correlation (r_s); multilocus paternity correlation (rp_m); number of trees effectively pollinating (Nep); proportion of siblings by self-fertilization (PSS); proportion of half-siblings (PHS) and proportion of full-siblings (PFS).

RESULTS

The mean estimates of all parameters analyzed for the reproductive system of *M. scabrella* in the population were compiled and indicated significant variations between consecutive reproductive events of 2015 and 2016 (Table 1).

Table 1. Averages of reproductive system parameters of *Mimosa scabrella* in the study, referring to the reproductive events in 2015 and 2016.

Tabela 1. Médias dos parâmetros do sistema reprodutivo de *Mimosa scabrella* no estudo, referentes aos eventos

Parameter	Reproductive event			
	2015		2016	
	average	CI	average	CI
Multilocus outcrossing rate (t_m)	0,925*	0,914-0,936	0,845*	0,835-0,855
Single locus outcrossing rate (t_s)	0,783*	0,767-0,799	0,675*	0,665-0,685
Outcrossing rate among relatives ($t_m - t_s$)	0,142*	0,130-0,153	0,170*	0,162-0,178
Self-fertilization rate (s) = $1 - t_m$	0,075*	0,064-0,086	0,155*	0,148-0,165
Self-fertilization correlation (r_s)	0,070*	0,028-0,113	0,160*	0,130-0,190
Multilocus paternity correlation (rp_m)	0,204*	0,181-0,277	0,315*	0,293-0,338
Number of trees effectively pollinating (Nep): $1 / rp_m$	4,90*	4,542-5,263	3,17*	2,913-3,430
Proportion of siblings by self-fertilization ($PSS = s$): $1 - t_m$	0,075*	0,064-0,086	0,155*	0,148-0,165
Proportion of half-siblings (PHS): $t_m \cdot (1 - rp_m)$	0,736*	0,709-0,763	0,579*	0,559-0,599
Proportion of full-siblings (PFS): $t_m \cdot rp_m$	0,189*	0,169-0,209	0,266*	0,249-0,283

CI Confidence Interval: lower and upper limit for 95% confidence level; * significant difference to 5% probability between averages in the same line.

DISCUSSION

The multilocus crossing rates (t_m) were considered of high magnitude in the reproductive events of 2015 and 2016 in the study population (Table 1) and within expected standards for *M. scabrella*. Analyzing this same species with isoenzymatic markers, Moreira *et al.* (2011) obtained estimates of 0.780 and 0.832 for the parameter (t_m) in localities of the state of Santa Catarina, while Sobierajski *et al.* (2006) obtained estimates between 0.859 and 1,000 in populations in the states of São Paulo, Paraná and Santa Catarina. The high magnitude for the crossing rates (t_m) obtained are also in accordance with Sebbenn (2003), who considered that the high values for this parameter in leafy tree species are frequent, exemplifying the t_m average 0.913 for a group of 24 leafy trees. According to Karasawa (2009), the mixed reproductive system includes crossing rates (t_m) between 0.05 and 0.95, and in trees with mixed system also occur high rates.

With the high rates (t_m) of the study, it is inferred that there was gene recombination during the reproduction of 2015, a positive fact for maintaining the genetic variability of the species in the population. The inference is in line with Gusson *et al.* (2006), for which the recombination of genes can mean greater evolutionary potential for tree species and face natural selection in the environments where they occur. The average estimates (t_m) suggest a decrease in crosses in the reproductive event of 2016 in relation to the rate of 2015. The average values were significantly different, but the magnitude was not changed.

It is not ruled out that the environmental factor influenced the reproduction of the species after the 2015 reproductive event and reflected in the decrease in the multilocus crossing rate in 2016. Climatic factors such as wind and heavy rainfall in the formation phases of the floral buds and anthesis can alter the microenvironment of reproduction (the canopy). The breaking of branches and falling inflorescences, depending on the proportions, can affect the availability of food resources to pollinators and their behavior. Adverse conditions can alter the usual behavior of pollinating bees by decreasing or interrupting foraging and making them perform tasks of lower energy expenditure in the hive. For Silva *et al.* (2013), changes in temperature, luminosity, relative humidity (> 70%) and strong winds (> 2 m/s) can change the frequency of bee foraging. Mori *et al.* (2013) consider that the variation in the behavior and density of pollinators can generate differences in the crossing rate of tree species.

The estimates obtained for this parameter (t_m), for *M. scabrella* in 2015 and 2016, indicate that the proportions of crosses occurred are close to rates of species with predominance of allogamy. This fact is favorable for a species with hermaphrodite flowers, representing greater possibilities of genetic variability being maintained. The inference is in agreement with considerations by Manoel *et al.* (2012), for whom crosses favor the maintenance and expansion of genetic diversity of species in populations, due to the recombination of genotypes.

The single locus crossing rate (t_s) in the 2016 reproductive event was significantly lower than in the 2015 reproductive event (Table 1). According to Gusson *et al.* (2006), the occurrence of single locus crossing rate lower than the multilocus crossing rate may be associated with the lack of self-incompatibility mechanisms in tree species with hermaphrodite flowers. This situation occurred in the two reproductive events analyzed, confirmed by the existence of self-fertilization rates (s) and proportions of siblings by self-fertilization (PSS).

The outcrossing rate among relatives ($t_m - t_s$) increased significantly in the 2016 reproductive event compared to 2015 (Table 1). In population genetics, the increase in crosses between related individuals is not favorable to the species. In this study, it indicated that more individuals with kinship exchanged alleles among themselves in the 2016 reproductive event compared to the previous year, suggesting an increase in inbred crossings. In the hypothesis of continuity of this trend following reproductive events, there is the future possibility of lower randomness in the exchange of alleles at crossings and of potentiating inbreeding.

In populations of *M. scabrella*, the seed bank in the soil forms near each matrix tree, due to seed dispersal by autochory. For Sobierajski *et al.* (2006) and Sebbenn (2003), this fact increases the probability of "daughter plants", from various reproductive events, contributing to the formation of genetic structure in the population by crossing stems between related individuals, reflecting greater inbreeding.

The self-fertilization correlation (r_s) showed an estimate of low magnitude in the reproductive event of 2015, suggesting the same trend of low proportions of progenies generated by self-fertilization in the population. Because there is a value for this parameter (r_s), even low, demonstrates that spontaneous self-fertilization occurred, suggesting that there are no mechanisms of self-incompatibility in *M. scabrella*. The inference is supported by the evaluation of Sobierajski *et al.* (2006), when they concluded that estimates were low in nine populations of this species ($0.013 \leq r_s \leq 0.310$).

A significant increase in this parameter (r_s) was verified in the 2016 reproductive event (Table 1), which can be evaluated as unfavorable to the study population, suggesting that a higher proportion of siblings were generated by self-fertilization in the aforementioned period. By remaining the tendency to increase the correlation of self-fertilization in the next reproductive events, there is the possibility of reflecting unfavorably on inbreeding and genetic variability of the species.

The multilocus paternity correlations (rp_m) in the consecutive reproductive events analyzed can be considered of average magnitude, based on the evaluation of Sobierajski *et al.* (2006) for values of this parameter, such as 0.153 (average) and 0.694 (high), in populations of *M. scabrella*. Estimates (rp_m) show that 20.4% and 31.5% of the progenies generated in this population, respectively in 2015 and 2016 (Table 1), originated from non-random crosses, involving the same pairs of donor individuals and pollen recipients, generating complete sibling descendants. The deduction is in line with considerations by Mori *et al.* (2013), when they stated that the multilocus correlation of paternity is indicative of systematic crossings between the same parent pairs. In the 2016 reproductive event, the average parameter (rp_m) increased significantly in relation to the previous year's average, reflecting the higher occurrence of biparental crossings in the mentioned period.

Analyzing the results of the crosses, it is possible to infer that these were not exclusively random, because, in addition to the half-sibling descendants, full-siblings were also generated. The inference corroborates statements by Antiqueira and Kageyama (2015), that it is apparently common for crosses in tree species not to produce only half-sibling descendants.

The number of trees effectively pollinating (Nep) in the reproductive events of 2015 and 2016 is consistent with the standard observed for *M. scabrella*. The values obtained were not high, but proved to be sufficient to ensure that the crossings occurred at high rates, in both periods analyzed (Table 1). Moreira *et al.* (2011) evaluated populations of the same species in the state of Santa Catarina and the average of 4.38 pollinating trees was considered of low magnitude. The decrease in the estimate in the 2016 reproductive event, compared to 2015, does not favor the species in the population, as this parameter is a quantifier of pollen donor individuals in crosses. The result suggests the possibility that some factor influenced pollination in the reproductive period of 2016.

Braga and Collevatti (2011) analyzed consecutive reproductive events of *Tabebuia aurea* (Silva Manso) Benth. & Hook. and found variation in the number of pollen donor trees between the years evaluated, in the same population. It is possible to deduce that such a parameter (Nep) can oscillate in a population, influenced by factors that undergo changes in reproductive periods, including the amount of flowers produced and the abundance of pollinators of the species.

In the 2016 flowering in the study population, canopy of some matrices sampled with entirely dry branches were observed, as well as in others not included in the sample, suggesting a reduction in the fruit productive area. Although not quantified in the study, there is the possibility that this partial canopy drought, not seen in individuals in 2015, is an indicator of the onset of senescence in these, because it is a kind of short cycle and that there are no signs of disease or pests in them. The inference corroborates information from Ferreira *et al.* (2009), who observed some pioneer tree species with visible signs of senescence at age 13, such as death of branches, defoliation and bark release, demonstrating vital decline, similar to those observed in *M. scabrella*.

It is not ruled out that this occurrence also contributed, in part, to reduce the supply of food resources to pollinators in the 2016 reproductive event and influenced their foraging behavior, reflecting the reduction of this parameter (*Nep*). The deduction corroborates the considerations of Braga and Collevatti (2011), that variations in flowering can interfere in the number of pollen donor trees.

The proportion of siblings by self-fertilization (*PSS*), obtained in the 2015 reproductive event, represents the generation of 7.5 individuals by self-fertilization for every 100 descendants, while in the 2016 reproductive event 15.5 individuals were generated by self-fertilization for every 100 descendants (Table 1). This significant increase (106.6%) shows to be unfavorable to the species in the population due to the possibility of greater inbreeding. The deduction corroborates the statement of Gonçalves and Piña-Rodrigues (2007) that self-fertilization is the extreme way of generating inbreeding, suggesting reduction of heterozygotes for gene recombination.

Despite the aforementioned increase in 2016, the proportions of siblings by self-fertilization (*PSS*) had a low magnitude for both reproductive events analyzed. The inference was based on results for the same species, such as 0.194 (average populations) and 0.141 (highest estimate among populations), obtained respectively by Moreira *et al.* (2011) and Sobierajski *et al.* (2006), who evaluated them as low proportions.

The proportion of half-siblings (*PHS*) decreased in the 2016 reproductive event, compared to the same period of 2015, with a significant difference (Table 1). The decrease observed in 2016 can be analyzed as an unfavorable indicator in relation to randomness in crosses in this period, suggesting a smaller amount of heterozygotes for gene recombination.

It is not ruled out that some local and seasonal factor, external to the plant, such as heavy rainfall and wind, influenced the behavior of pollination vectors of *M. scabrella*, altering the routine of visitation to the flowering in the reproductive period of 2016. This deduction corroborates considerations by Antiqueira and Kageyama (2015) that pollinators can visit trees less randomly in search of food, when they are affected by environmental factors or by aspects inherent to the species itself.

The proportion of full-siblings (*PFS*) was significantly higher in the 2016 reproductive event than in the previous reproductive event (Table 1). It is considered that the significant increase in the estimation of this parameter *PFS* is not genetically interesting to the species in the population, suggesting that biparental crosses were more frequent in 2016, to the detriment of random crosses. The increase in the estimation of this parameter *PFS* suggests a higher concentration of pollinators to forage few trees, successively visiting the same parents and a reduction of random crosses. The proportions of half-siblings (*PHS*) and full-siblings (*PFS*) descendants, generated in the two reproductive events of this study, reinforce this possibility. It is inferred, corroborating considerations of Antiqueira and Kageyama (2015), who attributed the occurrence of biparental crosses to the behavior of pollinators, when they prioritize systematic visits in neighboring trees.

Even with the changes in the estimates from one year to the next (2015-2016), the expectation for the proportions of descendants of a species with hermaphrodite flowers (*M. scabrella*) was confirmed, with a greater number of half-siblings (*PHS*), followed by full-siblings (*PFS*) and with lower proportions of self-fertilization siblings (*PSS*).

CONCLUSIONS

- It is concluded that the species *M. scabrella* has a mixed reproductive system, with a predominance of crossings in the population.
- The crossings in the population of *M. scabrella* occur at high rates and most of the descendants generated by crossings are half-siblings;
- The occurrence of self-fertilization in *M. scabrella*, proves that the species does not have mechanisms of self-incompatibility;
- The number of pollen donor trees for each matrix of *M. scabrella* is not high and it is variable, but guarantees crosses predominantly and the continuity of survival of the species at each reproductive event;
- Even though there are significant differences between reproductive event parameters of *M. scabrella* in the years 2015 and 2016, the characterization of the mixed system for the species was maintained.

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