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Breeding system and pollination of *Pleroma trichopodum* DC. (Melastomataceae): a potential species for the restoration of Atlantic Forest in southern Brazil

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ABSTRACT

Plant-pollinator interactions and their reproductive implications are of central importance to the organization of plant populations and communities in restoration areas. We studied the breeding system and flower visitors of *Pleroma trichopodum*, a pioneer species of the Atlantic Forest. We attempted to answer three questions: (1) Is *P. trichopodum* dependent on pollinators and mates for reproduction? (2) What are the pollinators of *P. trichopodum*? (3) Do tree flower-density and flowering-tree density of *P. trichopodum* enhance the visitation rate of focal trees and their flowers? We tested the breeding system through pollination treatments. We performed focal observations on 10 trees and analyzed the relationship between tree and flower visitation rates, and the tree flower-density and flowering-tree density with Generalized Linear Mixed Models (GLMM). *Pleroma trichopodum* sets fruits by selfing and outcrossing, both of which occur only through pollinator visits. Bees visited *P. trichopodum,* and their visitation increased with tree flower-density. Thus, individuals with greater floral displays may function as magnet species, enhancing the pollination of nearby plant species. This characteristic, allied with the ability to reproduce without a mate (selfing) and a tolerance of soggy soils, make *P. trichopodum* a candidate for Atlantic Forest restoration.

Keywords: Atlantic Forest, breeding system, floral density, pioneer, plant-pollinator interactions, *Pleroma trichopodum*, restoration, selfing

Introduction

Plant-pollinator interactions and the reproductive outcome of such mutualisms are central to plant population and community organization (Ashman *et al.* 2004). For this reason, these topics have been intensively studied in a variety of contexts (Ayasse & Arroyo 2011). Nonetheless, key questions remain unanswered and, for several ecosystems, information is still lacking regarding the reproduction and pollination of most plant species, thus hindering the application of such frameworks to restoration and conservation (Rodrigues *et al.* 2009; Mayer *et al.* 2011; Menz *et al.* 2011).

The restoration of degraded areas is an alternative way of recovering the functionality of ecological systems (Young 2000), and has been used to restore ecosystems threatened by human activities, such as the conversion of tropical forests into pastures (Aide *et al.* 2000; Cheung *et al.* 2009). A high proportion of angiosperms depend on animals for their reproduction (Ollerton *et al.* 2011), while plant persistence is constrained by the breeding system (Bond 1994; Brys &

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Jacquemyn 2010). In this way, studies that integrate plant species pollination and breeding systems are of paramount importance for revealing insights on the persistence of plants in different environments and the identification of species with conservation and restoration potential (e.g. Aguilar *et al*. 2006; Neuschulz *et al*. 2016).

It is believed that the presence of generalist plants, with abundant rewards, is important for the establishment and maintenance of the natural processes of restoration (Dixon 2009; Menz *et al*. 2011). In general, plants that display a greater number of flowers have higher visitation rates (Klinkhamer & Jong 1990; Mitchell *et al*. 2004; Grindeland *et al*. 2005; Makino & Sakai 2007; Brys & Jacquemyn 2010). Nonetheless, a greater number of flowers might mean a reduction of, or no effect on, the visitation rate of each individual flower (Klinkhamer & Jong 1990; Mitchell *et al*. 2004; Grindeland *et al*. 2005). Likewise, a high density of flowering individuals in an area might lead to enhanced visitation for each due to facilitation (Kunin 1997; Thompson 2001; Moeller 2004; Makino & Sakai 2007; Brys & Jacquemyn 2010; Hegland 2014). Nonetheless, high densities of individuals that overlap in flowering, both in time and space, might cause competition for pollinators, leading to decreased visitation rates per individual (Mustajärvi *et al*. 2001; Sargent & Ackerly 2008; Hegland 2014).

Plants in initial restoration areas may become isolated from conspecifics and suffer from a low availability of mates (Scobie & Wilcock 2009). Moreover, the degradation of the environment in early restoration areas may limit the availability of pollinators (Kremen *et al*. 2007). In this sense, plants that reproduce through *selfing* have the advantage of reproductive assurance (Pannell & Barrett 1998; Moeller & Geber 2005; Santos *et al*. 2012), but may suffer disadvantages related to high inbreeding rates (Lande & Schemske 1985; Herlihy & Eckert 2002; Maia *et al*. 2017). On the other hand, self-incompatible plants avoid inbreeding (Lande & Schemske 1985), but may be unsuccessful in areas with limited availability of pollinators and mates (Pannell & Barrett 1998; Scobie & Wilcock 2009). Thus, plants with a mixed breeding system**,** i.e. involving self-pollination and cross-pollination, and that produce abundant floral resources and attract a great diversity of pollinators, are expected to be good candidates for use in restoration programs

The family Melastomataceae comprises 4,200–4,500 species and is well represented in tropical and subtropical ecosystems of the Americas, where there are approximately 3,000 species (Renner 1993; Goldenberg *et al*. 2012). Melastomes have been reported as key species for restoration practices due to their seed and seedling ecology (Silveira *et al*. 2013). In addition, melastomes are important pollen sources for many bee species (Harter *et al*. 2002). Such bees are adapted to perform buzz pollination, which enables the extraction of pollen by sonication (Buchman 1983) to feed their larvae (Michener 2007). The floral morphology of some species of Melastomataceae, such as the occurrence of poricidal anthers and herkogamy (Renner 1989), suggests that these species do not perform autonomous self-fertilization, and require pollinators for pollen transfer.

The present study investigated the breeding system and pollination biology of *Pleroma trichopodum* DC. (Melastomataceae) (Fig. 1), which, until recently, was named *Tibouchina trichopoda* (Meyer *et al*. 2010). *Pleroma trichopodum* is a pioneer tree adapted to environments saturated with water (Meyer *et al*. 2010; Maia *et al*. 2013), and is abundant in areas undergoing early natural regeneration in southern Brazil (Cardoso 2014; Warring *et al*. 2016). Although it has potential for use in the restoration of Atlantic Forest, knowledge of its reproductive and pollination biology is incomplete, particularly regarding its pollinators and mating system. For instance, it is known that large floral displays by this species are related to enhanced reproductive success (Maia *et al*. 2013), but whether this is associated with increased floral visitation is unknown.

Figure 1. *Pleroma trichopodum*. **A.** Flower in detail, showing the poricidal anthers. **B.** A tree with a large floral display.

Individuals of *P. trichopodum* occurring in natural regeneration areas were selected to address three major questions: (1) Is *P. trichopodum* dependent on pollinators and mates for reproduction? (2) What are the pollinators of *P. trichopodum*? (3) Do tree flower-density and flowering-tree density of *P. trichopodum* enhance the visitation rate of focal trees and their flowers?

Materials and methods

Study area

This study was conducted between September 2009 and February 2010 in areas undergoing early succession in the Guaricica Natural Reserve (25°18'50"S 48°41'45"W). This reserve is located within an environmental protection area (Área de Proteção Ambiental de Guaraqueçaba) in Antonina, on the northern coast of Paraná State, southern Brazil. The climate is subtropical humid, with an average annual precipitation of 2534.5 mm (Vanhoni & Mendonça 2008) and an annual average temperature between 20.8 °C and 22 °C (Ferretti & Britez 2006).

Breeding system and floral visitors

From September 2009 to November 2009 we performed five pollination treatments (Radford *et al*. 1974): open pollination, in which we labeled buds in pre-anthesis and left them without isolation; autonomous self-pollination, in which we labeled buds in pre-anthesis and isolated them to exclude pollinators; manual self-pollination, in which we transferred pollen manually from one tree to the stigma of flowers of the same tree, isolating them after this procedure; outcrossing, in which we transferred pollen from different trees to the stigma of flowers of a focal tree, isolating the flowers after this procedure; apomixis in which we removed the styles and stamens of buds in pre-anthesis before isolation. We surveyed the treatments throughout one month to assess fruit formation in each of the surveyed trees. The number of flowers varied among treatments, but the minimum was 70. We used eight trees for the open pollination and autonomous self-pollination treatments and seven trees for the manual self-pollination, outcrossing and apomixis treatments. We calculated fruit set as the ratio between the number of ripened fruits and the number flowers in each treatment. We calculated the independence of fruit set among treatments by Pearson's Chi-squared test using R software (R Core Team 2017). We calculated the Index of Self-Incompatibility (ISI, *sensu* Bullock 1985) as the fruit-set ratio between manual self-pollination and outcrossing treatments; ISI values higher than 0.25 were interpreted as an indication of selfing (Zapata & Arroyo 1978). We calculated Reproductive Efficacy (RE; Zapata & Arroyo 1978; Oliveira & Gibbs 2000) as the ratio between fruit-set from the open pollination treatment and fruit-set from the outcrossing treatment. Along with the reproductive tests, we performed opportunistic floral visitor observations as well as focal observations in different individuals to assess the complete floral visitor guild. Floral visitors were collected and sent to specialists for identification.

Visitation rate as a function of floral display

To determine how tree visitation rate (visits per hour on each tree) and flower visitation rate (visits per hour per flower on each tree) varied as a function of the density of flowering trees next to focal trees and the density of flowers displayed by the focal trees, we performed observations on 10 flowering individuals of *P. trichopodum* from November 2009 to February 2010. The focal trees were distributed among six young natural regeneration sites with trees in the same being separated by at least 30 meters. These young successional areas possessed a great abundance of exotic grasses that usually dominated the wetter portions (Ferretti & Britez 2006; Cardoso 2014). In fact, many of the individual trees we observed were completely surrounded by these grasses. During four months, we performed approximately 10 hours of focal observations for each focal plant for a total of 100 hours of observation. We observed each plant in two bouts, one in the morning (five hours) and one in the afternoon (five hours), but not necessarily on the same day due two logistics and weather conditions. We counted the number of flowering *P. trichopodum* plants in a circular plot of 15 m of radius (706.5 m^2) around the focal trees to assess the density of flowering trees. To determine the density of flowers in each focal tree we marked an area of 1.20 x 1.20 m (1.44 m^2) on the north and in the south faces of the plants crown in which we counted the number of flowers and observed pollinator visitation. We switched between north and south face of the crown every 30 minutes of observation.

All floral visitors were collected and sent to specialists for identification. Only visitors that acted as potential pollinators were included in the analysis. We considered as potential pollinators visitors that contacted the flower stigma when collecting pollen from the anthers and visitors that, when releasing the pollen from single anthers, created a pollen cloud that could eventually reach the stigma (Maia *et al*. 2016). We considered visitors that collected pollen without touching the stigmas as pollen thieves. The number of visits was summed for each tree in each bout. Since each tree was observed during two bouts (one morning and one afternoon), we ended up with 20 replicates. The number of visits during each replicate was divided by five hours to calculate the visitation rate per hour at each tree in a given bout (tree visitation rate). Additionally, we divided this visitation rate by the number of flowers counted on each tree during an observation bout to calculate the flower visitation rate on trees (flower visitation rate). Data for visitation rate, tree flower-density, flowering-tree density, hours of observation and observation bout are available as Table S1 in supplementary material.

To assess the effect of focal tree flower-density and flowering-tree density on tree visitation rate and flower visitation rate, we used Generalized Linear Mixed Models (GLMM), assuming a normal error distribution, performed with the *lme4* package (Bates *et al*. 2015). We used tree visitation rate and flower visitation rate as response variables, tree flower-density and flowering-tree density as fixed predictors, and sites and bout as random factors. Thus, we could account for any lack of independence among replicates at the same site or during the same bout. We scaled the predictor variables (tree flower-density and flowering-tree density) prior to the analysis. We assessed the significance of tree flower-density and flowering-trees density by likelihood ratio tests when dropping each variable from the full model (Campos-Arceiz *et al*. 2008; Turrini & Knop 2015), using the *car* package (Fox & Weisberg 2011). All the analyses were conducted with the R software (R Core Team 2017).

Results

Pleroma trichopodum produced fruits by selfing and outcrossing and did not produce fruits by apomixis and autonomous selfing (Tab. 1). Fruit set was similar among control, selfing and outcrossing treatments ($X^2 = 0.17$; df = 2; $p > 0.05$; Tab. 1). The high values for the auto incompatibility index (ISI) suggest selfing for *P. trichopodum*, while the high values for reproductive efficacy suggest a high efficacy of natural pollination (Tab. 1).

Table 1. Fruit set (sample size) of the four reproductive treatments for *Pleroma trichopodum*. ISI - Index of Self-Incompatibility. RE - Reproductive efficacy.

Treatment	Fruit set (number of flowers)
Open pollination	52.5(80)
Autonomous self-pollination	0(70)
Manual self-pollination	54.7(75)
Outcrossing	50.7(73)
Apomixis	0(71)
ISI	1.08
RE	1.04

Only bees were recorded on *P. trichopodum* flowers, eleven of which were pollinators and one of which was a pollen thief (Tab. 2). The behavior of bees of the genera *Bombus*, *Centris*, *Thygater* and *Xylocopa* was similar. These bees landed on the flower, embraced several anthers and vibrated them, releasing a pollen cloud characteristic of buzz-pollination. The bees of the genus *Augochloropsis* vibrated one anther at a time, releasing a pollen cloud that eventually could contact the stigma (Maia *et al*. 2016). Bees of the genus *Tetragonisca* behaved as pollen thieves, collecting pollen by chewing the top of the anthers.

Flower density of the focal trees ranged from 4.17 to 95.49 flowers per m^2, while the flowering-tree density ranged from 0 to 0.06 trees per m^2. The GLMM analysis showed that tree visitation rate was significantly affected by tree flower-density but not by flowering-tree density (Fig. 2A, B, Tab. 3). The relationship between tree visitation rate and tree flower-density was strongly positive (Fig. 2A, Tab. 3). Nonetheless, neither of the two-predictor variables affected flower visitation rate (Fig. 2C, D, Tab. 3).

Table 2. Floral visitors of *Pleroma trichopodum* identified as pollinators or pollen thieves.

Species	Visitation type
Augochloropsis sp. 1	pollinator
Augochloropsis sp. 2	pollinator
Augochloropsis sp. 3	pollinator
Augochloropsis sp. 4	pollinator
Bombus (Fervidobombus) brasiliensis (Lepeletier, 1836)	pollinator
Bombus (Fervidobombus) morio (Swederus, 1787)	pollinator
Bombus (Fervidobombus) pauloensis (Friese, 1913)	pollinator
Centris (Aphemisia) mocsaryi (Friese, 1899)	pollinator
Tetragonisca angustula (Latreille, 1811)	pollen thief
Thygater sp.	pollinator
Xylocopa (Neoxylocopa) brasilianorum (Linnaeus, 1767)	pollinator
Xylocopa (Neoxylocopa) frontalis (Olivier, 1789)	pollinator

Table 3. Results of the Generalized Linear Mixed Model using visitation rate per tree and per flower as response variables, tree flower-density and flowering-tree density as fixed predictors, and sites and bout as random factors. The significance of the predictors was assessed by likelihood ratio test (LRT), considering values of *p* lower than 0.05 as significant. Significant variables are in bold.

	GLMM		Likelihood ratio test	
	Coef.	SE	LRT	p
Tree visitation rate				
Intercept	0.640	0.137		
Tree flower-density	**0.402**	**0.099**	**11.519**	**< 0.001**
Flowering-tree density	0.005	0.102	0.002	0.964
Flower visitation rate				
Intercept	0.009	0.003		
Tree flower-density	-0.004	0.003	1.879	0.170
Flowering-tree density	0.001	0.003	0.027	0.870

Discussion

Pleroma trichopodum is completely dependent on pollinators for reproduction, which are attracted by large floral displays. Nonetheless, the possibility of self-pollination reduces the necessity of mates for its reproduction.

The strong effect of tree flower-density on tree visitation rate shows that, for *P. trichopodum*, the investment of energy into big floral displays is a successful strategy for attracting pollinators. The benefit of larger floral displays is associated with the cognition ability of bees (Schiestl & Johnson 2013), for which visual cues might enhance their efficiency (Kunze

& Gumbert 2001). The lack of a relationship between tree visitation rate and density of flowering conspecifics could be related to the absence of competition or facilitation. Another possibility is that both processes occur at a same time. If the latter is true, the lack of a pattern in the analysis could be due to the fact that one pattern confounds the other, thus precluding the identification of either. Although an enhanced display of flowers attracted more pollinators to focal trees, this was not related to variation in visitation rate per flower (Mitchell *et al*. 2004), as previous detected for other species (Klinkhamer & Jong 1990; Grindeland *et al*. 2005). In this way, we did not confirm that enhanced fruiting success in *P. trichopodum* plants with more flowers, as detected in a previous study (Maia *et al*. 2013), could be due to enhaced visitation of pollinators to each flower.

Most of the bee species that were found attracted to *P. trichopodum* are common for species of the Melastomataceae genus *Tibouchina* (genus to which *P. trichopodum* belonged until recently; Pereira *et al*. 2011; Franco *et al*. 2011; Brito & Sazima 2012). Several floral visitors of one species of *Tibouchina* (*T. pulchra*), studied by Pereira *et al*. (2011), belonged to three genera also found visiting *P. trichopodum*, and vary in their relative lengths (size). This trait variation could indicate a certain degree of functional complementarity and enhance the reproductive success of plant communities (Fontaine *et al*. 2006; Albrecht *et al*. 2012). The enhanced attractiveness of high floral display plants to different bees shows that these trees might function as islands of resources (Brito *et al*. 2015) in areas where exotic herbs are dominant. Although we could not see a clear pattern of facilitation, by attracting many pollinators with different pollination traits,

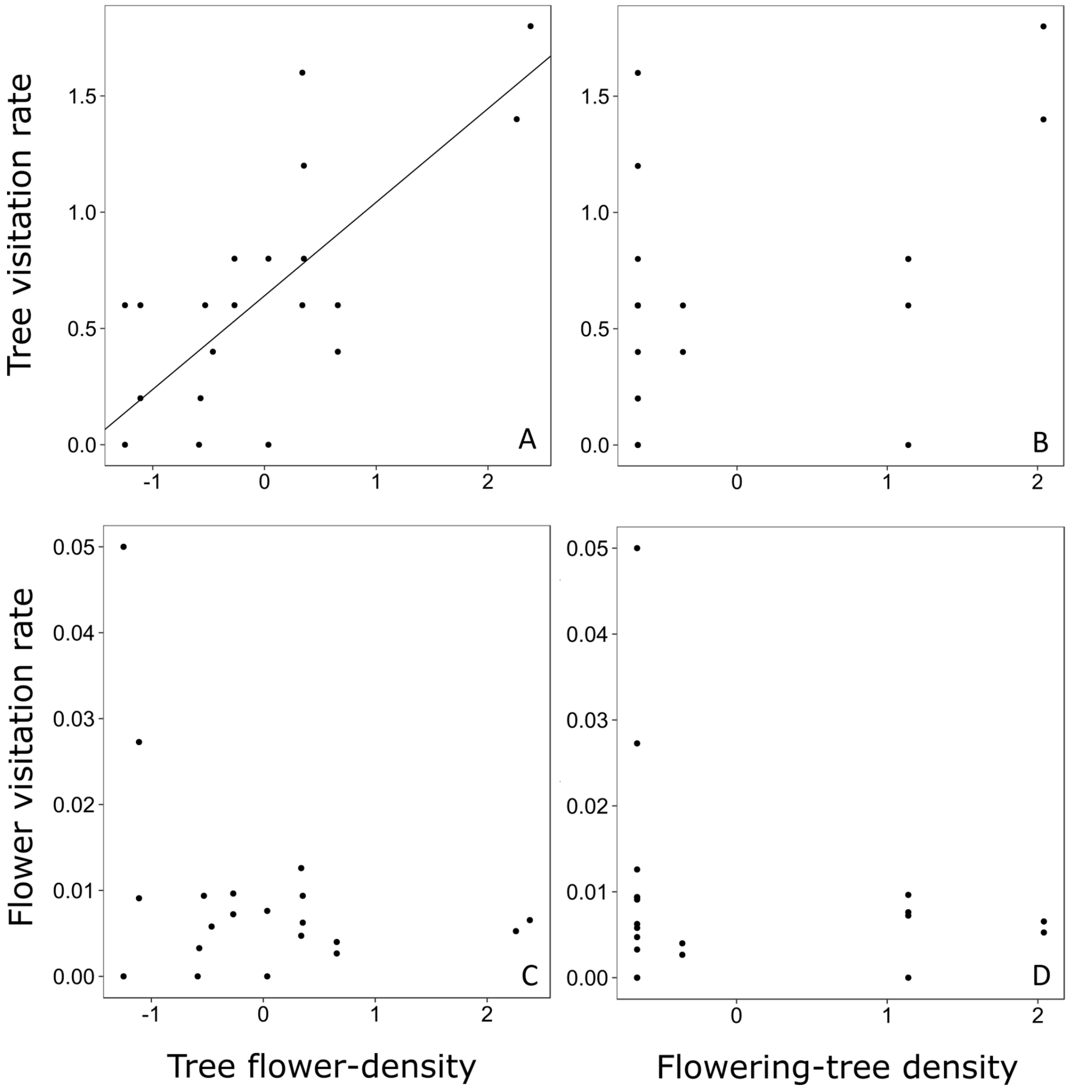

Figure 2. Relationship between tree visitation rate and tree flower-density (**A**) and flowering-tree density (**B**). Relationship between flower visitation rate and tree flower-density (**C**) and flowering-tree density (**D**). Tree visitation rate is the number of visits per hour on each tree in a given bout of focal observation. Flower visitation rate is the tree visitation rate divided by the number of flowers of each tree in a given bout.

these plants might enhance the chance of pollination for neighbor plants. It is known that the presence of isolated trees in abandoned pastures facilitates the establishment of other species, by reducing competition with exotic grasses and by enhancing the arrival of seeds that are dispersed by animals (Zwiener *et al*. 2014). Here we argue that *P. trichopodum* might also provide pollinator assurance to plants that are growing under its canopy or in its proximity, thus functioning as a magnet species (Molina-Montenegro *et al*. 2008). Furthermore, previous studies with *P. trichopodum* report two flowering seasons: an extended flowering period with scattered flowering individuals during the less rainy season; and a brief flowering period with a high density of flowering individuals during the hot, rainy season (Maia *et al*. 2013). In this way, *P. trichopoda* might also be considered a bridging plant since it provides floral resources over a long temporal span and in periods of the year with contrasting climates (Dixon 2009).

Pleroma trichopodum reproduces by a mix of selfing and cross-pollination. The high value of the ISI index indicates that self-pollination by this species is highly efficient, which is common among other species of Melastomataceae (Goldenberg & Shepherd 1998; Franco *et al*. 2011; Pereira *et al*. 2011; Maia *et al*. 2016). The independence from mates for the exchange of pollen for reproduction through selfing, confers this plant with a good capacity to colonize new areas (Holsinger 2000), which is reflected in its presence in early succession areas (Cardoso 2014). Nonetheless, autonomous selfing does not occur, probably because of the spatial separation between the anthers and the stigma (herkogamy), and because of the tiny pore of the anthers (Renner 1989). Thus, *P. trichopodum* is entirely dependent on bee pollination for its reproduction. This dependence might lead to limited pollination in areas where the availability of bees is low (Brito & Sazima 2012). Still, the high reproductive efficacy (RE) suggests that these bees are efficient pollinators and relatively abundant and active in our study area.

The possibility of selfing and the attraction of a diverse guild of pollinators, as well as the adaptation to wet soils, show that *P. trichopodum* has potential for use in the assisted restoration of areas where the soil remains soggy most of the time (Ferretti & Britez 2006). Since tree visitation rate increases with the number of flowers on the tree, but not with tree density, spaced planting of vigorous or more developed individuals is a better strategy to attract pollinators to an area than planting clumped small or young individuals. These sparse individuals can act as stepping-stones and increase pollen dispersal between different areas (Kitamoto *et al*. 2006).

Our study provided valuable biological information on an abundant pioneer species of the Atlantic Forest of southern Brazil. *Pleroma trichopodum* revealed characteristics of a good colonizer that deserves further study for use in restoration purposes. By studying bee visitation rate on individual plants, we reinforced the hypothesis that these insects are mostly attracted by the abundance of floral resources. Nonetheless, for *P. trichopodum* the effect of conspecific plants remains unanswered.

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