

Original Paper

Two dioecious *Simarouba* species with a specialized pollination system and low reproductive efficacy in Central Brazil

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Abstract

Several possible correlations between dioecy and eco-morphological features have been suggested to infer the conditions that would favor this sexual system. Dioecy has been associated either with specialized or generalized pollination systems. The genus *Simarouba* has six dioecious species that seem to have generalized pollinators. In this study, we examined the reproductive biology aspects of two *Simarouba* species to evaluate which of their eco-morphological attributes may favor dioecy. Our data suggest that *S. amara* and *S. versicolor* have small, inconspicuous, and abundant flowers of nocturnal anthesis and are only pollinated by nocturnal moths. They have a stable sexual system with flower, inflorescence, and flowering phenology dimorphism that is part of their pollination system strategies. Male plants of both species produce more flowers per individual than do female plants. A larger floral display may have an essential role in increasing male reproductive success. However, both species showed low reproductive efficacy, especially *S. versicolor*. The low reproductive efficacy may be due to the lack of pollinators or to the inefficacy of the pollinators available locally.

Key words: Cerrado, dioecy, moth pollination, *Simarouba*, Simaroubaceae.

Resumo

Várias correlações entre dioécia e características ecomorfológicas têm sido sugeridas para inferir as condições que favorecem este sistema sexual. A dioécia tem sido associada a sistemas de polinização especializados ou generalistas. O gênero *Simarouba* possui seis espécies dióicas que parecem ter polinizadores generalistas. Neste trabalho, examinamos aspectos da biologia reprodutiva de duas espécies desse gênero para investigar quais de seus atributos ecomorfológicos reprodutivos podem favorecer a dioécia. Nossos dados sugerem que *S. amara* e *S. versicolor* têm flores pequenas, abertas, abundantes, com antese noturna que são polinizadas apenas por mariposas também noturnas. Elas possuem um sistema sexual estável com dimorfismo na flor, inflorescência e fenologia de floração, que faz parte de suas estratégias de polinização. As plantas masculinas de ambas as espécies produzem mais flores por indivíduo do que as femininas. Esta alta produção de flores tem um papel importante no aumento do sucesso reprodutivo masculino. Ambas as espécies apresentaram baixa eficácia reprodutiva, especialmente *S. versicolor*. A baixa eficácia reprodutiva pode ser devido à falta de polinizadores ou à ineficácia dos polinizadores disponíveis localmente.

Palavras-chave: Cerrado, dioécia, polinização por mariposa, *Simarouba*, Simaroubaceae.

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Introduction

The flower is an organ that has several complex structures and is adapted to sexual reproduction (Endress 1995). Most plant species have hermaphroditic flowers, and about 5–6% of Angiosperms are dioecious with staminate and pistillate flowers on separate plants (Renner 2014). Dioecy has probably evolved several times within angiosperms from hermaphroditism (Ross 1982), via gynodioecy (Charlesworth & Charlesworth 1978), monoecy (Dorken & Barret 2004), or heterostyly (Darwin 1877; Lloyd 1979). Selective forces for the evolution of this sexual system involve genetic and ecological mechanisms (Bawa 1980a; Givnish 1982; Thomson & Barret 1981). The sex separation between male and female plants requires cross-breeding between different individuals and can bring genetic advantages to the population (Charlesworth & Charlesworth 1978; Freeman *et al.* 1997) and improve individuals' reproductive fitness (Bawa 1980a; Thomson & Barret 1981). On the other hand, these plants become dependent on pollination vectors (Oliveira & Maruyama 2014). As a consequence of this sexual separation, staminate and pistillate flowers have secondary sexual characteristics, such as differences in size and attractiveness, which are considered adaptive strategies that allow the directional flow of pollen to co-specific stigmas (Grant 1995; Lloyd & Webb 1977).

Some authors argued that ecological constraints are more important than selection for outcrossing in dioecy evolution (Givnish 1982; Bawa 1980b; Willson 1994). Sexual specialization would optimize the resource allocation for reproduction because male and female functions would demand different resources in distinguished ways (Moore & Pannell 2011; Tonnabel *et al.* 2017). The male function would be involved mainly with the production and dispersion of pollen to increase the availability of mating partners, and the female function would invest in ovule and fruit production, optimizing the quality of seeds (Janzen 1977; Bawa 1980a; Lloyd 1982).

Several possible correlations between dioecy and various eco-morphological features have been suggested to infer the conditions that would favor this sexual system (Bawa 1980a; Renner & Feil 1993; Renner & Ricklefs 1995; Renner 2014). According to Bawa (1980a), flowers of dioecious species are generally small and unspecialized, have light and inconspicuous colors, and are produced in more significant numbers in male than in female

plants. This sexual dimorphism is interpreted as one of the consequences of sexual selection to increase an individual's sexual display and promote male reproductive success (Bawa & Opler 1975; Bawa 1980b). Some other authors supported the ecological association of dioecy with small insect-pollinated flowers (Givnish 1982; Thomson & Brunet 1990). However, these correlations pointed out by Bawa (1980a) were contested by Renner & Feil (1993), who considered the occurrence of dioecy to be more common in plants with more specialized flowers. Few field observations about sexual systems in natural populations show which eco-morphological attributes favor dioecy (Renner 2014).

The genus *Simarouba* Aubl. has six tree or shrub species, all of which are dioecious, occurring in Cerrado, Amazonian Forest, and Dry forest of Central America and Caribbean Island (Franceschinelli *et al.* 1999). All genus species are truly dioecious, but other closely related genera, such as *Simaba* Aubl., particularly section *Tenuiflorae*, also show signs of dicliny (Alves *et al.* 2017). The six species of *Simarouba* have distribution ranges restricted to the Neotropical region (Clayton 2011), with two of them, *S. amara* Aubl. and *S. versicolor* A. St.-Hil, occurring in Brazil (Devecchi & Pirani 2016). Some studies emphasized the phenology and reproductive biology of *S. amara* (Pinto *et al.* 2005). Dioecy was reported as a sexual system for this species, with pollination performed by several small insects (Bawa 1994; Hardesty *et al.* 2005). Pollination by generalist bees has been suggested for *S. versicolor* (Reis *et al.* 2012). These works reinforce the theory that dioecious species generally have more generalized flowers and pollinators.

Simarouba amara Aubl. has a broad geographical distribution, occurring from Central America to Southeast Brazil. *Simarouba versicolor* A. St.-Hil. occurs from North to Southeast Brazil and in Bolivia (Pirani & Thomas 2015). Both species can co-occur and co-flower in the Cerrado biome. However, regardless of the wide-ranging area of occurrence, data on these species' reproductive systems are still scarce or inconclusive.

In this context, the objective of this work was to expand knowledge about the sexual system and pollination of *Simarouba* species that occur in Brazil. We intended to examine both floral and pollinating attributes related to dioecy. Specifically, we evaluate whether these species' pollination is

performed by small and diverse insects, considering Bawa's (1980a) premises, or if their flowers are specialized to be pollinated by a specific group of pollinators as suggested by Renner & Feil (1993) for most dioecious species. We expect that both species have small and generalist flowers pollinated by diverse insects, such as suggested by other authors (Bawa 1994; Hardesty *et al.* 2005; Reis *et al.* 2012). We also expect that both *Simarouba* species have low reproductive efficacy, and the staminate flowers are produced in a larger amount than pistillate ones, as suggested by Bawa (1980a) for dioecious species.

Material and Methods

We conducted the study from July to October 2015 and 2016 for *S. amara* Aubl., and June to November 2016 for *S. versicolor* St.- Hil in the Santuário de Vida Silvestre Vagafogo, in the municipality of Pirenópolis, state of Goiás, Brazil (15°49'00''S, 48°59'30''W). The average altitude of the locality is 750 m above sea level, and the local vegetation is characterized as a fragment of seasonal semideciduous forest and cerrado (Venturoli 2008).

Study species

Simarouba amara is a tree species (Fig. 1a) that can reach up to 35 m in height in the Amazon Forest's *terra firme* forests, where it can be quite common species. This species also inhabits the Cerrado biome at the southern end of its geographical distribution (Raw & Hay 1985; Franceschinelli *et al.* 1999). It is also a common species in different phytophysiognomies of Cerrado, especially in Cerradão and Cerrado *sensu stricto* of its Central Brazil distribution (Raw & Hay 1985; Franceschinelli *et al.* 1999). *Simarouba versicolor* is also a tree species (Fig. 2a), but it inhabits mainly Cerrado (*sensu lato*) and the Caatinga biomes (Pirani & Thomas 2015). These species were reported to be of pharmacological, commercial, and ecological importance (Souza 1997; Trevisan & Macedo 2003; Cortes 2012).

Flowering phenology, floral morphology, and biology

We described the morphology of flowers and inflorescences using ten pistillate and ten staminate plants of both species (five inflorescences and five flowers per analyzed plant). For each species, we registered the opening time, the emission of odor,

pollen grain release in 10 staminate and 10 pistillate flowers of 10 individuals of each sex. We evaluated stigmatic receptivity in the field by adding a few drops of 3% H₂O₂ solution to the stigma of flowers (Kearns & Inouye 1993). We estimated male fertility by analyzing pollen viability. We collected five flowers in pre-anthesis from five individuals and fixed them in 50% FAA for 24h, transferring them to 70% alcohol after that. We prepared slides by staining pollen grains with 2% acetic carmine. We visualized all pollen grains we found on the slides using an optical microscope at 10% magnification and counted with the aid of a manual counter.

We performed phenological analyses of *S. amara* and *S. versicolor* using 20 individuals of each species (ten males and ten females), which we marked and identified during the flowering period. We made weekly observations of flower production during the reproductive period of each species. We quantified the flowering phenophase by assigning points that designated an approximate value to its intensity (Fournier 1974). We also calculated the flowering phenophase activity index between male and female flowers and between the studied species (Bencke & Morellato 2002).

Sex ratio and display of staminate and pistillate flowers

We determined the sex ratio from the number of male and female individuals that were reproductive in one study population per species. We used a Chi-square test to assess the ratio (1:1) of male and female individuals using R (R Core Team 2015). We evaluated the species' sexual pattern during their reproductive period of 2015–2016 for *S. amara*, and 2016–2017 for *S. versicolor*, and we observed whether the plants would remain dioecious and with the same sex from one reproductive period to the other.

We counted the number of flowers of four branches located on the plant's opposite side from five male and five female individuals to analyze flower production investment in plants from both sexes. Using a digital caliper with 0.01 mm accuracy, we also performed a morphometric analysis of staminate and pistillate flower petals of 100 flowers (50 staminate flowers and 50 pistillate flowers) from five plants of each sex for *S. amara*. We performed the same analysis for *S. versicolor* using 60 flowers (30 staminate flowers and 30 pistillate flowers) from five plants of each sex. Finally, we used an independent t-test to determine

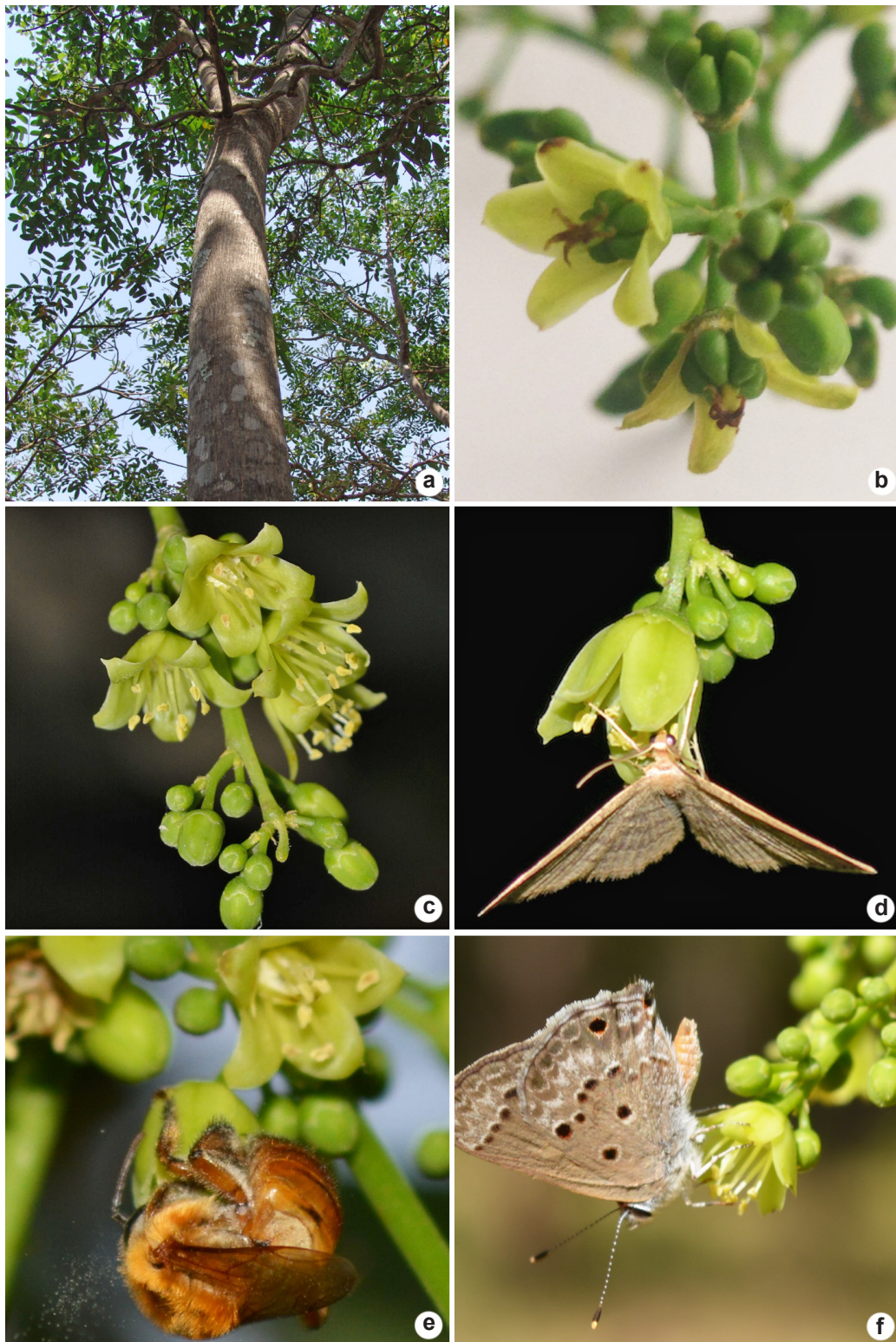


Figure 1 – a-f. *Simarouba amara* in Santuário de Vida Silvestre Vagafogo, Pirenópolis, GO, Brazil – a. tree of *S. amara*, 14 m tall; b. pistillate flowers; c. staminate flowers; d. *Pleuroprucha insularia* visiting a staminate flower of *S. amara*; e. *Melipona rufiventris* taking pollen out of a staminate flower in pre-anthesis; f. the butterfly *Echynargus isola* visiting a staminate flower of *S. amara* in the morning.

any differences in the average flower production and the average size of staminate and pistillate petals and inflorescences.

Floral visitors

We observed the behavior of the floral visitors for both species at different times of the day and night during six non-consecutive days of the flowering peak, under low air humidity (20 to 35%), warm to mild temperature (34 to 17 °C), and mild wind (lower than 3 km/h). We observed during the whole day and night, totaling 84 hours

of observation for each species. We collected data on the frequency of pollinators' visits in three male and three female plants of one population for each species through focal observation for two hours (number of pollinators visits / 2h) of observation during three nights. We considered pollinators those animals that touched the stigmas of the pistillate flowers and the anthers of the staminate flowers when visiting them to collect the floral resources (Alves-dos-Santos *et al.* 2016). We analyzed the body of each insect with the aid of a stereomicroscope to assess the presence of pollen

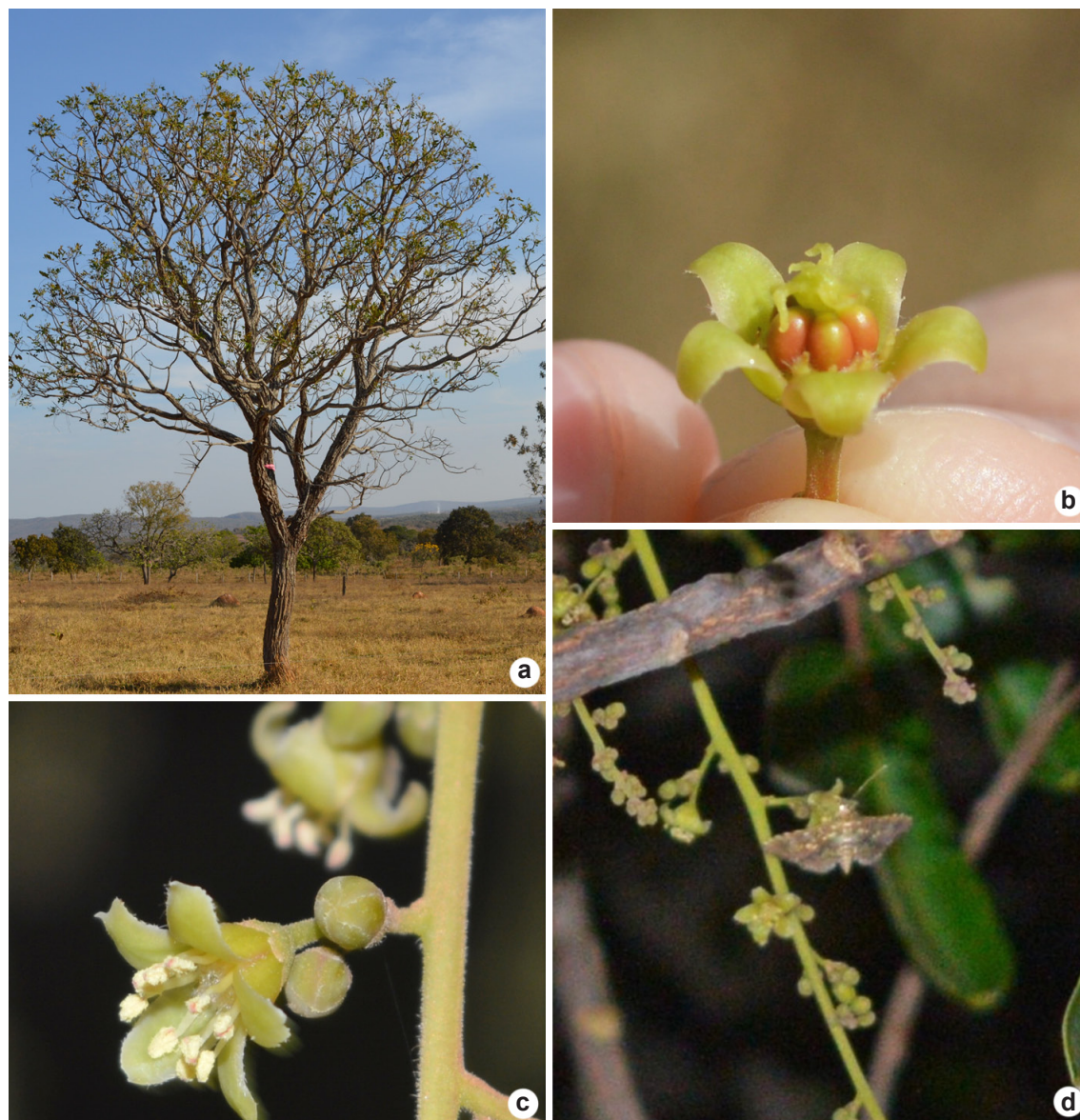


Figure 2 – a-d. *Simarouba versicolor* in Santuário de Vida Silvestre Vagafogo, Pirenópolis, GO, Brazil – a. tree of *S. versicolor*, 8m tall; b. pistillate flower; c. staminate flowers; d. Noctuidae in a staminate flower of *S. versicolor*.

grains. We compared blades with pollen grains found on the insects with the pollen grains of *S. amara* and *S. versicolor* flowers under an optical microscope. We identified the insects and deposited them in the Coleção Entomológica of Laboratório de Biologia Reprodutiva Vegetal of Universidade Federal de Goiás.

Reproductive system and efficacy

We performed the following experiments: manual cross-pollination (when we carried pollen from the anthers of staminate flowers to the stigmas of pistillate flowers from previously bagged inflorescences); apomixis (when we marked and bagged pistillate flowers in pre-anthesis); open pollination (when we marked pistillate flowers but did not bag them); and a test to check for wind pollination (when we bagged pre-anthesis pistillate flowers using a tissue with a large pore size that allowed the pollen grains to get into the bag). We performed each procedure on a minimum of 30 flowers from five different plants per treatment.

We used organza tissue bags measuring 10×10×14 cm in the manual cross-pollination and apomixis experiments. This material was closed enough to prevent visitors' entrance and allow ventilation for flower's and fruit's development. In the wind pollination treatment, we used micro-perforated fabric bags to prevent insect visitation but permit the passage (entry) of pollen grains into the bags. We compared fruit yield between open pollination (OP, control) and manual cross-pollination (CP) to determine reproductive efficacy (RE) (modified from Freitas 2013):

$$OP = \frac{\text{n}^\circ \text{ of fruits produced by open pollination}}{(\text{n}^\circ \text{ of flowers} \times 5 \text{ carpels})} \times 100$$

$$CP = \frac{\text{n}^\circ \text{ of fruits produced by cross-pollination}}{(\text{n}^\circ \text{ of flowers} \times 5 \text{ carpels})} \times 100$$

$$RE = \frac{OP}{CP}$$

We multiplied the number of flowers by five because each flower has five apocarpous carpels that can develop up to five drupoid fruits.

Results

Flowering phenology, floral morphology, and biology

Simarouba amara and *S. versicolor* have greenish-yellow unisexual flowers arranged in terminal or axillary panicles. The terminal or axillary

panicles of *S. versicolor* are pendulous on male plants and erect on female plants. In *S. amara*, the male and female panicles are erect, making it easy to distinguish the male plants of both species during the flowering period. Both species have actinomorphic, pentamerous, gamosepalous, and dialypetalous flowers. The pistillate flowers (Figs. 1b; 2b) have a superior, pentacarpellate, and apocarpous ovary with the presence of a nectariferous gynophore and ten rudimentary stamens composed of pilose filaments and reduced non-functional anthers. The style is gynobasic, and the stigma is pentalobate. The staminate flowers (Figs. 1c; 2c) also have a nectariferous gynophore with reduced carpels and are diplostemonous. The anthers are basifixed with longitudinal dehiscence. The pistillate flowers can produce up to five independent drupes measuring 0.3–1.5 × 0.2–1.0 cm for *S. amara* and 2.0–2.5 × 1.5–2.0 cm for *S. versicolor*. They are very dark in color, close to black, and take around 50 days to mature.

In both species, flowering started in July and ended in September (Fig.3), showing simultaneous flowering (Fig. 3c), although *S. amara* started flowering a week earlier and finished a week later than *S. versicolor*. The period of greatest flowering intensity occurred in middle August for *S. amara*, remaining in flower until the end of September. For *S. versicolor* flowering peak was at the end of August and beginning of September, ending abruptly in the middle of September. There was more significant overlap between the male and female flowering period in *S. versicolor* than in *S. amara*, in which staminate flowers start open in abundance a week or ten days before pistillate flowers (Fig. 3a-b).

For both species, staminate flowers opened in the late afternoon, between 17:15h and 18:00h. Pistillate flowers open between 18:00 to 20:00 for *S. amara* and 19:30 to 21:00h for *S. versicolor*. However, on very hot days (36 °C or more), the opening of staminate flowers began around 16:30h.

The pistillate flowers of *S. amara* and *S. versicolor* and the staminate flowers of *S. amara* remained open for nearly two days while the staminate flowers of *S. versicolor* expired and dropped within 24 hours. For both *S. amara* and *S. versicolor*, open pistillate flowers presented stigmas receptive with a clear and bright appearance for 12 hours. After that, stigmas were slightly dark and did not bubble in the 3% H₂O₂ solution, indicating that they were no longer receptive. Therefore, the stigma seems to be receptive during the night and in the following early morning only.

Pollen started to be available in staminate flowers one to two hours after the buds began to open and remained functional during the night. In the morning, the amount of pollen on both species' anthers is very low or null. Ninety percent of the pollen grains analysis from pre-anthesis flowers of *S. amara* and *S. versicolor* had their cytoplasm stained with acetic carmine and considered viable on the same day of anthesis. Flowers of *S. amara* and *S. versicolor* produced a mild odour that we could sense only in the early evening when the flowers were in the anthesis process.

Sex ratio and display of staminate and pistillate flowers

The proportion of female (n = 41) and male (n = 42) flowering plants was 1:1 ($\chi^2 = 0.31$; p = 0.58) for *S. amara* and for *S. versicolor* (female, n = 14 and male n = 17, $\chi^2 = 0.29$; p = 0.59). There was no change in the sex of individuals for during the study period for both species.

The average number of staminate flowers/inflorescence for *S. amara* ($X = 4048.30 \pm 3223.12$) was 10.8 times higher than that of pistillate flowers ($X = 392.20 \pm 138.04$; t = 2.53, p = 0.03). The results were similar for *S. versicolor*, with the

number of staminate flowers being 10.3 times higher ($X = 1836.75 \pm 288.64$) than that of the pistillate flowers ($X = 169.25 \pm 20.61$; t = 16.29, p < 0.01). Besides, petals of staminate flowers of *S. amara* ($X = 4.26 \pm 0.5272$ mm) were larger than those of pistillate flowers ($X = 4.06 \pm 0.2399$ mm; t = 4.80; p < 0.01). We observed the opposite for *S. versicolor*, with pistillate flower petals ($X = 3.78 \pm 0.31$ mm) being larger than those of staminate flowers ($X = 2.87 \pm 0.42$ mm; t = 6.97; p < 0.01).

Floral visitors

We observed Noctuidae, Crambidae, Geometridae, and Uraniidae moths on both pistillate and staminate flowers of *S. amara* during the night, mainly between 18:00–23:00h (Tab. 1; Figs. 1d; 2d). We observed Crambidae and Geometridae moths visiting flowers of *S. versicolor* mostly between 19:00 to 23:00h (Tab. 1; Fig. 1d). All observed moths touched the anthers of staminate flowers and the stigma of pistillate flowers during their visits to both species' flowers. We found pollen grains on the ventral part, the antennae, and the proboscis of all moths (N = 9 for *S. amara* and N = 7 for *S. versicolor*) collected both in pistillate and staminate flowers. These pollen grains were collected and observed on slides under de microscopy.

The visits of the moths to staminate flowers of both species started at nightfall and became more intense between 21:00 and 22:00h. To pistillate flowers, the visits occurred mainly between 21:00 to 23:00h and were rarer than for male plants for both species, especially for *S. versicolor*. In *S. amara*, we saw 1–3 moths/hour visiting around 10–15 male flowers/plant and two moths/hour visiting 5–7 female flowers/plant. In *S. versicolor*, 0.5–1 moths/hour visiting around 6–12 male flowers/plant and 0.5 moth/hour visiting 3–5 female flowers/plant.

Diurnal insects were rarely seen visiting pistillate flowers of both *Simarouba* species and were not considered effective pollinators. At the end of the afternoon, we observed bees visiting the staminate flowers mainly for pollen collection in both species (Tab. 1; Fig. 1e). They started visiting flowers of male individuals by 17:00h, remaining until nightfall. They got the rest of the pollen from the *S. amara* and *S. versicolor* old staminate flowers and, sometimes, nectar from *S. amara* extrafloral nectaries. Eventually, we observed *Apis mellifera* Linnaeus 1758 and *Melipona rufiventris* Lepeletier, 1836, forcing anther opening of pre-anthesis flowers to get pollen.

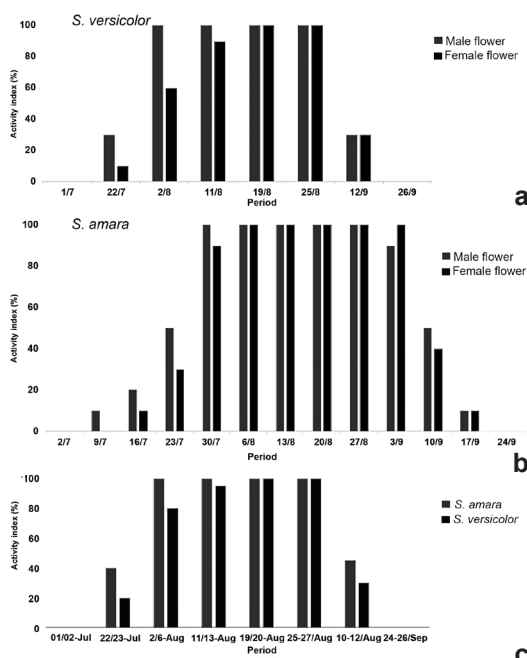


Figure 3 – a-c. Activity index (%) of the flowering phenology – a. *Simarouba amara*; b. *S. versicolor*; c. between both species.

Table 1 – The nocturnal pollinators and diurnal visitors of flowers of *Simarouba amara* and *Simarouba versicolor* (Pirenópolis GO, Brazil).

Species	Nocturnal Pollinators	Diurnal Visitors
	Lepidoptera	Hymenoptera
<i>S. amara</i>	<i>Mamestra brassicae</i> L (Noctuidae)	<i>Melipona rufiventris</i> Lepeletier, 1836 (Apidae: Meliponini)
	<i>Epidromia pannosa</i> Guenée 1852 (Noctuidae)	<i>Trigona</i> sp. (Apidae: Meliponini)
	<i>Pleuroprucha insularia</i> , Guenée 1857 (Geometridae)	<i>Frieseomelitta varia</i> Lepeletier 1836 (Apidae: Meliponini)
	Noctuidae 1	<i>Apis mellifera</i> Linnaeus 1758 (Apidae: Apini)
	Crambidae 1	Wasp
	Crambidae 2	
	Crambidae 3	Lepidoptera
	Crambidae 4	<i>Echynargus isola</i> (Lycaenidae)
	Geometridae 1	
	Uranidae	
<i>S. versicolor</i>	<i>Mamestra brassicae</i> L (Noctuidae)	
	<i>Pleuroprucha insularia</i> , Guenée 1857 (Geometridae)	
	Noctuidae 1	
	Crambidae	

Early in the mornings, bees of *Trigona* sp., butterflies of *Echynargus isola* (Lycaenidae) (Fig. 1f), and more rarely wasps visited staminate flowers of both species to collect nectar and the rest of pollen left on anthers at the end of the floral anthesis of both *Simarouba* species. We also observed ants and bees, such as *Tetragonisca angustula* Latreille 1811 (Apidae: Meliponini), getting nectar at the leaf extrafloral nectaries of male plants of *S. amara*.

Reproductive system and efficacy

The reproductive efficacy was low for both species. The flowers subjected to the cross-pollination treatment had a higher fruit production rate than did marked open flowers for both species (Tab. 2). However, flowers of *S. amara* subjected to the cross-pollination and control treatments had higher rates of fruit production than did flowers of *S. versicolor* subjected to the same treatments. Therefore, the reproductive efficacy (RE) was higher for *S. amara* than for *S. versicolor* (Tab.

2). In the apomixis experiment, few fruits were produced in both species (Tab. 2). We did not verify anemophilous fruit production for both species.

Discussion

Simarouba amara and *S. versicolor* have small, inconspicuous, and abundant flowers, which are common characteristics among tropical dioecious species and were associated with the attraction of small social bees, wasps, and flies pollinators (Bawa 1980a; Beach 1981). However, some floral characteristics of *Simarouba*, particularly pale open flowers with nocturnal anthesis and mild nocturnal odor, restrict its pollination to nocturnal visitors, such as small moths. These floral features may be related to phalaenophily syndrome (Faegri & Van der Pijl 1979). The small moths touch the anthers and stigmas of the staminate and pistillate flowers, respectively, while visiting flowers for nectar, when the stigmas are receptive, the anthers are properly open and full of pollen. These results corroborate

Table 2 – Reproductive efficacy and the fruit set (number of fruits / number of flowers) in open pollination, cross-pollination, wind pollination, and apomixis treatments for *Simarouba amara* and *Simarouba versicolor* (Pirenópolis GO, Brazil). (N) = number of flowers per treatment.

Species	Treatments				
	Open pollination (N)	Cross-pollination (N)	Apomixis (N)	Wind pollination (N)	Reproductive efficacy (RE)*
<i>S. amara</i>	7.97% (1771)	42.64% (106)	0.71% (1626)	0% (57)	18.7%
<i>S. versicolor</i>	1.35% (664)	20.80% (50)	0.61% (872)	0% (61)	6.5%

* see the text for detailed explanation

the theory of Renner & Feil (1993) that tropical dioecious species' pollination involves a more specialized pollination system.

The floral characteristics of *Simarouba* do not preclude the visit of other insects such as small bees in the daytime. However, diurnal insects were not effective pollinators because few pollen grains were available in the anthers of staminate flowers, and the visits to pistillate flowers were null or rarely observed in the daytime. Some bees sought pollen out of pre-anthesis staminate flowers that still have their anthers closed in the afternoon, visiting almost exclusively male plants for pollen. Besides, the female flowers did not have their stigma receptive in the afternoon, only in the night.

As suggested by Bawa (1980a) for dioecious species, the study species present low reproductive efficacy (RE = 18.7% for *S. amara* and 6.5% for *S. versicolor*), and male plants produce more flowers than female ones. These characteristics were previously reported for other dioecious species, including Cerrado species (Oliveira 1996; Freeman *et al.* 1997; Amorim & Oliveira 2006). A larger male floral display plays an important role in increasing the pollen amount in the system and the male reproductive success (Bawa 1980a). For *S. amara*, staminate flowers also presented larger petals, as previously observed for other dioecious species (Amorim *et al.* 2011; Franceschinelli *et al.* 2015a). Larger staminate flowers may occur when pollinator attraction confers a more significant gain in male than female reproductive success (Delph *et al.* 1996). In this case, male reproductive success would be conditioned by high flower production and pollen release, whereas female reproductive success would be conditioned by high fruit and seed production and maturation (Freeman *et al.* 1997). The female function is especially energy-intensive when the fruits are fleshy, and the seeds

are large (Bawa 1980a), such as for *S. amara* and *S. versicolor*. Furthermore, the high cost of reproduction for females may incur late flowering (Cipollini & Whigham 1994) and low pistillate flower production (Thomas & La Frankie 1993), as observed for the study species.

The staminate flowers of *S. versicolor* are smaller than the pistillate flowers. Perhaps, larger pistillate flowers are essential for increasing female floral display, as the number of flowers in female inflorescences of *S. versicolor* is lower than the inflorescences of *S. amara*. Pistillate flowers of larger petals may enhance female reproductive efficacy in *S. versicolor*, which is relatively low relative to *S. amara* and other dioecious species of the Cerrado (Fuzeto *et al.* 2001; Lenza & Oliveira 2005; Mendes *et al.* 2011).

The temporal separation of flowering between the sexes, such as observed mainly in *S. versicolor* is brief and should not cause damage to the pollination system. Early flowering of male plants should, at first, allow the initial attraction of pollinators and the recognition of flowers of this species as important resource donors (nectar). The available pollen grains should later be delivered to pistillate flowers due to the common morphological similarities between the flowers of different sexes of dioecious species (Bawa 1980b; Lenza & Oliveira 2006; Franceschinelli *et al.* 2015a). After a few days, when the pistillate flowers open, the pollinators recognize the available resource and the simultaneous flowering of plants of different sexes can provide greater pollen flow to female plants (Bawa 1980b; Bullock & Bawa 1981; Piratelli *et al.* 1998; Fuzeto *et al.* 2001; Lenza & Oliveira 2006).

Despite the specialized pollination system, a low reproductive efficacy was observed for both studied species, especially for *S. versicolor*. Some factors were cited as limiting pollinator

efficiencies in the open areas of the Cerrado, such as seasonal drought, high temperatures, and distance between plants of the same species (Oliveira 1996). The population density of *S. versicolor* was about a third of the density of *S. amara* (EV. Franceschianelli, unpublished data), which may restrict the genetic exchange between co-specific plants, especially if dioecious. The fruit set (7.97%) for *S. amara* from natural pollination was similar to the fruit set for *Virola sebifera* (5.8%) (Lenza & Oliveira 2006), lower than for *Tapirira guianensis* (23.5%) (Lenza & Oliveira 2005) and *Cabralea canjerana* subsp. *polytricha* (32%) (Fuzeto *et al.* 2001), but a little higher than for a few other dioecious species of Cerrado (Oliveira 1996).

The low reproductive efficacy of these species may be due to the low frequency of the visits of the moths to pistillate and staminate flowers, in particular for *S. versicolor*. However, other dioecious Cerrado species are also pollinated by moths, such as *C. canjerana* subsp. *polytricha* (Fuzeto *et al.* 2001) and *Amaioua guianensis* Aubl (Amorim & Oliveira 2006) have higher reproductive efficiencies than *S. amara*, although they also have low pollinator's visitation rates. Changes in the reproductive system of species may be common at the extremes of their distributions, where the primary pollinators may not be present and genetic diversity within populations may be smaller, increasing inbreeding and disrupting crossbreeding strategies (Barrett 1996; Reich *et al.* 2021). Also, we conducted the present study in a 5 km² Cerrado vegetation fragment with areas in different conservation states, which may also contribute to the low visitation rate of the pollinators to the studied flowers.

The data showed here demonstrate that the studied species are dioecious with various characteristics consistent with this sexual system, such as small, inconspicuous flowers and staminate plants producing a more considerable amount of flowers. The pollination system of *S. amara* and *S. versicolor* is performed specifically by nocturnal moths, but the populations studied revealed low reproductive efficacy. This may be due to the lack of pollinators in the area or the inefficiency of the available pollinators. Instead, small nocturnal moths may also be another group of pollinators that inefficiently pollinated dioecious species, such as Meliaceae species (Bawa 1990; Fuzeto *et al.* 2001; Mabblerley 2010; Franceschinelli *et al.* 2015b). Future studies with both species in

other better-conserved areas of Cerrado and the Amazon Forest (*S. amara*) could assess whether these observed characteristics and reproductive efficacy are consistent with their occurrence areas.

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