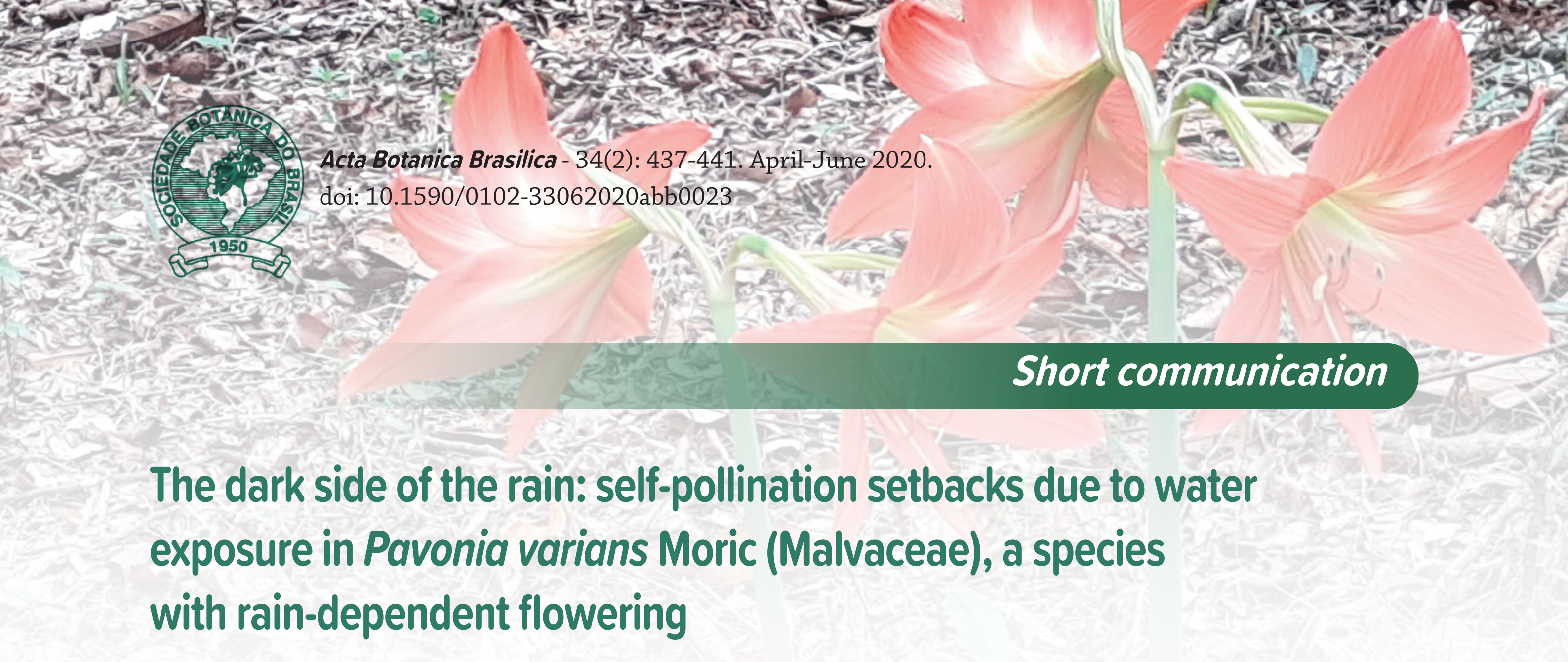

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Short communication

The dark side of the rain: self-pollination setbacks due to water exposure in *Pavonia varians* Moric (Malvaceae), a species with rain-dependent flowering

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ABSTRACT

Plants that occur in arid or semi-arid environments may restrict their flowering to the rainy season to ensure water availability for reproductive functions. However, the exposure of flowers to rainfall can compromise their functionality, such as complex self-pollination mechanisms. We experimentally tested how rainfall exposure affects the sequence of anthesis and reproductive success of *Pavonia varians*. This species is endemic to the Caatinga, and it has a rainfall-dependent flowering and a specialized mechanism of delayed self-pollination by style curvature. Our results demonstrate that wet flowers have difficulty in distending their petals, and although they maintain the sequence of anthesis, style curvature and corolla closure are delayed. Water exposure also compromised reproductive success by spontaneous self-pollination in bagged flowers but did not prevent fruit set by exposed flowers. These conditions allow cross-pollination in *P. varians* if rainfall is transient or localized, which is a common condition in the Caatinga. We believe that this type of experimental approach can bring important clues about how reproductive systems respond to abiotic factors, especially in the context of imminent climate change.

Keywords: autogamy, Caatinga, delayed self-pollination, floral anthesis, style curvature

Many angiosperms in arid and semi-arid environments restrict their flowering to the rainiest periods of the year (Machado *et al*. 1997; Struck *et al*. 1994). In fact, this condition ensures the water availability required for reproductive functions (Gallagher & Campbell 2017; Phillips *et al*. 2018); however, it exposes the flowers to the rain. This exposure may be a problem because it usually promotes detrimental effects on the interaction between plants and pollinators (*e. g.* pollinators avoiding to fly in the rain and reduction of floral attractiveness by enfeebled signage or nectar dilution), limiting cross-pollination events (Ruan *et al*. 2009; Lawson & Rands 2019). Besides that, rain can have other deeper effects, compromising the floral

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morphology and movements of the species, which can affect even mechanisms of self-pollination (Jacquemart 1996).

Plants in dry regions may resort to self-pollination in order to ensure reproductive security in response to environmental instabilities (Pedersen & Ehlers 2000; Evans *et al.* 2011; Naghiloo *et al.* 2019). In self-pollinating species, the sequence of anthesis interacts synergistically with floral morphology, often involving complex movements, which favors the arrival of pollen to the receptive stigmas (Lloyd & Schoen 1992; Domingos-Melo *et al.* 2018). This delicate adjustment can be greatly affected by rain, being rare the cases of rain-triggered self-pollination, in which the exposure of flowers to rain takes a beneficial aspect (Hagerup 1950; Daumann 1970; Suetsugu 2019). In most of the cases rainfall damages floral whorls and compromises pollen viability and stigmatic receptivity (Jacquemart 1996; Hase *et al.* 2006; Ortega *et al.* 2007; Mao & Huang 2009).

From field observations, we noticed that the rain seems to play these both roles (flowering promotion and floral damage) in *Pavonia varians* Moric (Malvaceae). This plant is a Caatinga endemic sub-shrub (BFG 2015), that grows and blooms massively at the beginning of the rainy season and the entire individual dies soon after fruiting. Despite its herkogamy promoted by staminal tube (Esteves 2000), we recorded in the field that *P. varians* has self-pollination through delayed style curvature (Fig. 1A-B). This is a common mechanism in Malvaceae, and was already reported for the genus *Pavonia* (Buttrose *et al.* 1977; McDade & Davidar 1984; Ruan *et al.* 2010; Goodwillie & Weber 2018; Ruiz-Guajardo *et al.* 2018). In this mechanism, the flowers show stigmas located above the anthers (Fig. 1A), characterizing hercogamy, during most part of the anthesis; but if there is no deposition of pollen on the stigma, the styles curve towards the anthers, coming in contact with the pollen and promoting self-pollination (Fig. 1B), as reported for some Malvaceae (Ruan *et al.* 2009; 2010; Goodwillie & Weber 2018; Ruiz-Guajardo *et al.* 2018). This easily observable and manipulable mechanism made *P. varians* a suitable model for performing our experiment. In our study, we conducted the experiments simulating the effect of rain on this autogamous system, and we seek to understand (i) how the rain affects the events of anthesis associated with self-pollination mechanisms, and (ii) how the rain affects the reproductive success of this plant species.

We performed the experiments on sunny days in April, 2016, with a population surrounded by shrubby and arboreal Caatinga dry forest on a sedimentary area in Catimbau National Park, Buíque - Brazil (8°34’20” S, 37°14’45” W). The climate of this park is semi-arid and there is a spatial precipitation gradient with average temperatures from 20 to 22 °C and mean annual rainfall from 510 to 940 mm (Rito *et al.* 2017). However, most of this rain is concentrated in 3 months year^{-1} usually varying between March and July, being the other months very dry, i.e. even less than 20 mm of rain per month (Cavalcanti & Corrêa 2014; Rito *et al.* 2017).

In order to test the effect of rain exposure to the anthesis sequence, we randomly took 15 *P. varians* individuals. In each of them, we marked two pre-anthesis buds. These buds were covered with voil bags in order to avoid the action of pollinators or florivores. The flowers from these bagged buds in each individual were exposed to two treatments: i) flowers under simulated rainfall; ii) control treatment, i.e. flowers without simulated rainfall (Fig.1C-D). In the first treatment,

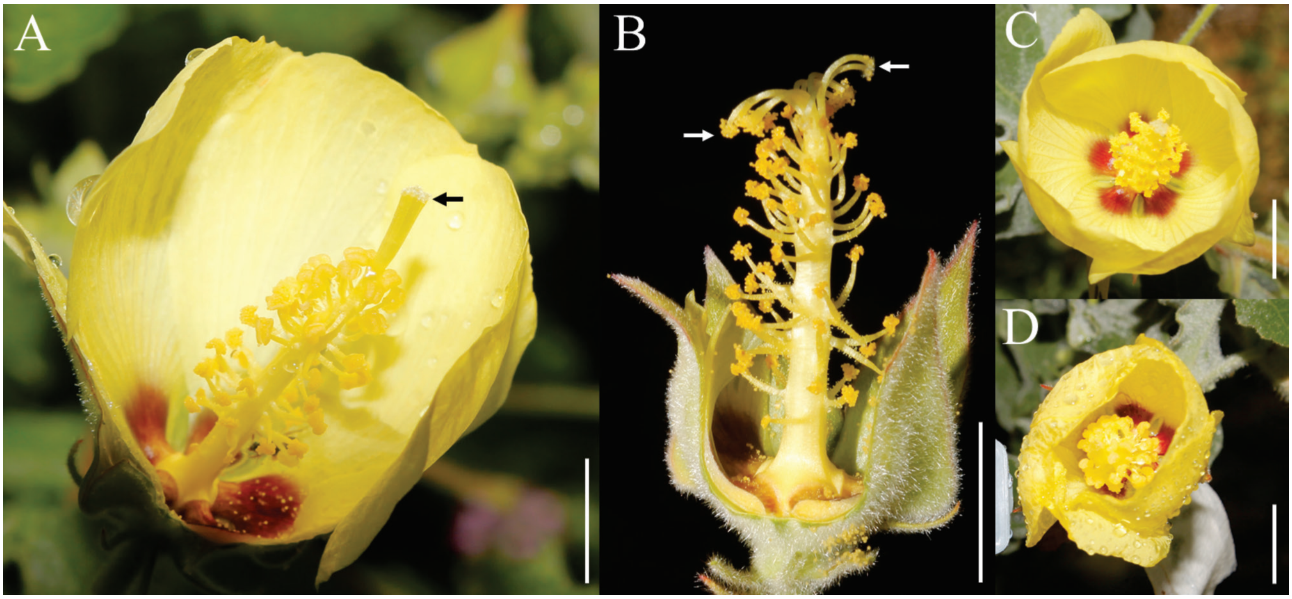

Figure 1. Floral anthesis and delayed self-pollination mechanism by style curvature in *Pavonia varians* at PARNA Catimbau, Pernambuco - Brazil. (**A**) Longitudinal section of a flower at anthesis with petals on maximum distension, (**B**) delayed self-pollination mechanism by curvature of the style, and top view of flowers of the same individual (**C**) without exposure and (**D**) after exposure to water. (black arrows - upward-facing stigmas on upright style; white arrows - downward-facing stigmas on curved styles; vertical white bars - 1 cm scale).

we did periodic rain simulations throughout floral anthesis, every 30 min from 6:00h to 18:00. For these simulations, we momentarily removed the voil bag and sprayed distilled water (~10 ml) for 20 seconds at a distance of 40 cm from the flowers (a configuration capable of replicating the apparent effect of 30 min of rain under natural conditions). In the control treatment, there was no manipulation. In both cases, the flowers were monitored from aperture to wilting time. We performed scaled photos of flowers in the upper view every 30 min, from these photos we measured the corolla diameter with ImageJ. We also recorded the time of the beginning of each of the four anthesis events: start of the floral aperture, the maximum opening of the floral aperture, the beginning of style curvature, and complete flower wilting. We performed quadratic regressions to describe floral aperture patterns and used paired t-tests to compare the time of the different anthesis events and the maximum corolla aperture reached.

In order to verify the effects of rain on fruit and seed set, we performed four treatments: i) spontaneous self-pollination in dry flowers, ii) spontaneous self-pollination in wet flowers, iii) natural pollination in dry flowers, and iv) natural pollination in wet flowers. For the autogamy treatments (i and ii), the flowers remained bagged throughout the anthesis, while in natural pollination (iii and iv) they remained freely exposed to the pollinators. In wet flower treatments (ii and iv), the flowers received the rain simulation as described above throughout the anthesis, while the dry flowers (i and iii) were not handled. Then, we checked for fruit and seed set. We compared the proportion of fruits between treatments through homogeneity chi-square tests, while for the number of seeds we used Mann-Whitney test.

The flowers of *P. varians* started opening at 6:30, already with the dehiscent anthers. The flowers reached maximum bell-shaped distention between 9:00 and 14:00. At this moment, the styles were kept erect (Fig. 1A). The styles began their curvature towards the anthers between 10:00 and 14:30, promoting spontaneous self-pollination (Fig. 1B). At this same interval, the flowers began to wilt, which was completed between 15:00 and 18:00. The aperture pattern of both dry and wet flowers was described by quadratic regressions (dry flowers: F = 61.06; df = 2; r^2 = 0.70; $p < 0.0001$ / wet flowers: F = 39.26; df = 2; r^2 = 0.56; $p < 0.0001$) (Fig. 2A), but the timing of anthesis events differed partially between dry and wet flowers (Fig. 2 B).

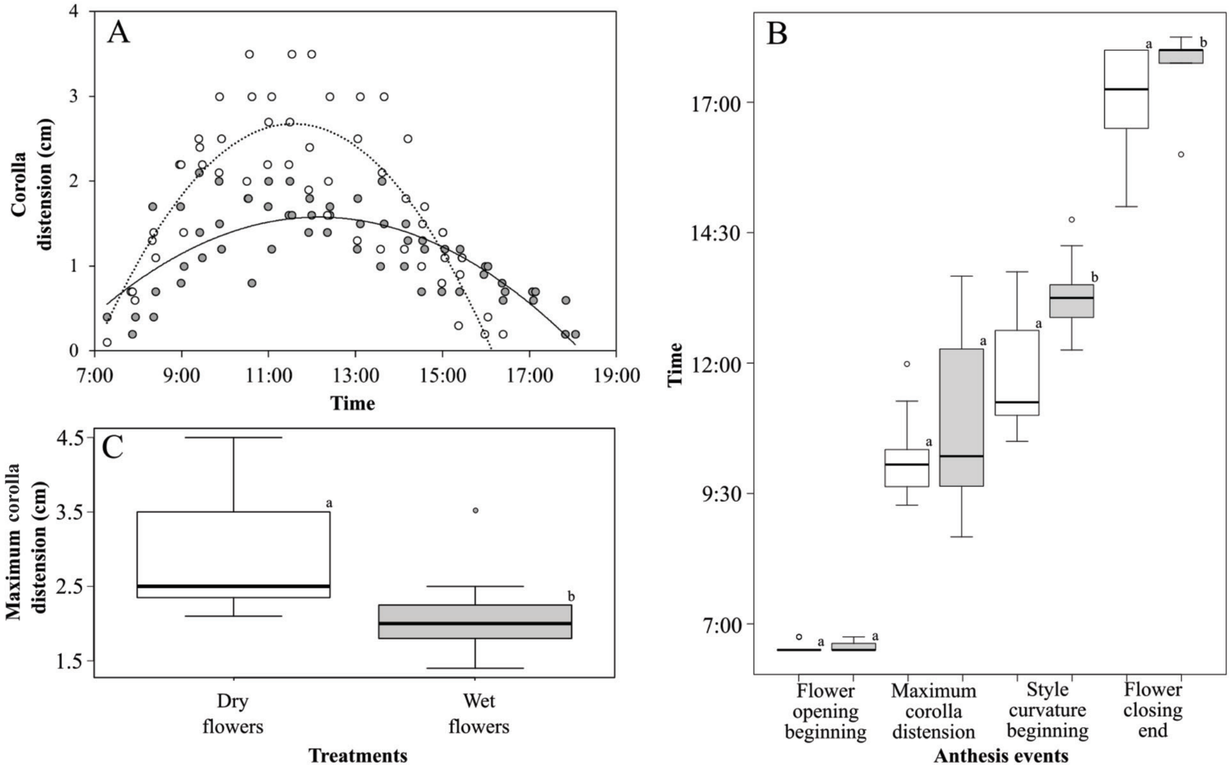

Figure 2. Differences between floral anthesis in wet flowers (treatment with rain simulation) and dry flowers (control treatment, without rain simulation) in *Pavonia varians* Moric (Malvaceae) at PARNA Catimbau, Pernambuco - Brazil. (**A**) Process of floral opening over time described by quadratic regressions. (**B**) Timing of the different events recorded during the anthesis. (**C**) Maximum corolla distension reached by the flowers. (gray color – wet flowers (with rain simulation); white color – dry flowers (without rain simulation); continuous line - trend line indicating the effect of time on the distention of the corolla in wet flowers; dotted line – trend line indicating the effect of time on the distention of the corolla in dry flowers; different letters in the box plots indicate significant difference with $\alpha < 0.05$).

Table 1. Effect of rain simulation on natural and autogamous fruit and seed set in *Pavonia varians* Moric (Malvaceae) at PARNA Catimbau, Pernambuco - Brazil.

	Water exposure	Fruit set		Seed set	
		count	%*	mean±sd	%**
Spontaneous self-pollination	Dry	13	(87 %) ^{a}	4±2	(71±37 %) ^{a}
	Wet	2	(13 %) ^{b}	3±0	(60±0 %) ^{a}
Natural pollination	Dry	14	(93 %) ^{a}	4±2	(74±31 %) ^{a}
	Wet	8	(53 %) ^{a}	3±1	(63±25 %) ^{a}

*Percentage of fruits per flowers, n = 15 flowers for each treatment.

**Percentage of seeds of each fruit in relation to the number of ovules per flower, flowers have five ovules each.

(Different letters indicate significant difference with p<0.05).

Dry flowers have a more abrupt aperture pattern (faster, larger floral apertures and with less variation among them) in relation to wet flowers. The dry flowers reached larger floral apertures when compared to wet flowers (t = 3.27, df = 14, p = 0.003) (Figs. 1C, 2C).

The beginning of the floral aperture was highly synchronized between the individuals and did not differ between the treatments (t = 1; df = 14; p = 0.1671) (Fig. 2B). Dry and wet flowers reached their maximum aperture at similar intervals (t = 1.73; fd = 14; p = 0.053) although the wet ones showed some retards. Wet flowers took a longer time than dry flowers to initiate style curvature (t = 6.37; gl = 14; p> 0.0001), retarding 1: 30 ± 0.55 h in relation to the dry flower of the same individual. Wet flowers also take a longer time than dry flowers to close (t = 2.19; df = 14; p = 0.0453), with a delay of 0: 39 ± 1: 08h.

The water can generate adhesion between the petals and between the styles, hampering the floral operation (Feng *et al.* 2011). In this sense, waterlogging of whorls can explain the retards recorded for some anthesis events. Although the curvature mechanism of styles strongly depends on synchronization with flower closure (Ruan *et al.* 2010; Goodwillie & Weber 2018), rain exposure did not compromise its occurrence.

Concerning the *Pavonia varians* reproductive success was high (Tab. 1). In turn, rainfall simulation negatively affected fruit set only through spontaneous self-pollination in comparison to other treatments (χ2 = 9.37, df = 3, p = 0.0248), but there was no effect on seed set (h = 3.27, df = 3, p = 0.3516).

The high reproductive success by autogamy in dry flowers demonstrates how the mechanism of delayed self-pollination by style curvature is efficient (Ruan *et al.* 2010; Domingos-Melo *et al.* 2018; Goodwillie & Weber 2018). However, the reproductive success by self-pollination in wet flowers was very low. This suggests that water might have a negative effect on the viability of pollen (Mao & Huang 2009). Thus, even if stigmas on the curved styles could contact anthers, pollen germination and fertilization of ovules would not occur (Ortega *et al.* 2007; Mao & Huang 2009).

Flowers exposed to the environment and rain simulations managed to form fruits even when they were wet. This probably occurred due to the action of pollinators that brought exogenous pollen, whose viability had not been compromised by water and that the stigmas of the flowers remain receptive and functional even under rain (Ortega *et al.* 2007). This condition can ensure that *P. varians* maintain a relatively high reproductive success in the case of local rains. Transient rainfalls may comprise from ~30 to ~70 % of total pluviometric events occurring in the Caatinga (Barbosa *et al.* 2018) and can occur in a very localized way, such as the orographic rainfall of Catimbau National Park (Cavalcanti & Corrêa 2014). Once *P. varians* is pollinated by bees, as the oligolectic bee *Ptilothrix plumata* Smith which collects pollen from this species (Schlindwein *et al.* 2009); *P. varians* flowers may receive pollinator visits after rain.

From an experiment with an endemic Caatinga species, we have shown that the same rain needed to evoke flowering can have detrimental effects on reproductive success, specifically in the case of a reproductive-safety mechanism such as spontaneous self-pollination. It is predicted rising temperatures and aridity for the coming years to naturally drier sites, among them the Caatinga (Li *et al.* 2018; Foden *et al.* 2019; Silva *et al.* 2019). In this scenario, we could expect that autogamy increasingly prevalent in species like *Pavonia varians*. It is urgent to know details of the abiotic processes that affect the reproductive biology of the plant in dry sites. In this context, the delicate balance between "rainfall dependence for flowering" and "damage to flowers and to reproductive systems caused by water" are important issues to be addressed in the current debate on the future of arid and semi-arid environments.

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