



STYLAR MOVEMENT IN *Talinum fruticosum* (TALINACEAE): CONTRIBUTIONS TO THE REPRODUCTIVE SUCCESS OF THE SPECIES IN POPULATIONS FROM CENTRAL-WEST BRAZIL

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Abstract: In plant species, the movement of reproductive structures can be an adaptive advantage in mating system studies. This phenomenon can promote outcrossing or cause a reduction in intrafloral male-female sexual interference. Consequently, this contributes to the avoidance of self-pollination and/or delayed autonomous self-pollination. The aim of this short communication is to report the results of assessments done in two natural populations of a native species from central-west Brazil that grows spontaneously and sub-spontaneously in opening and ruderal areas of Pantanal. In this study, the movement of the stigma was recorded throughout floral anthesis, and the mating system was determined. Floral measurements such as: flower opening time, pollen viability and the period that the stigma was receptive were also reported. The formation of fruits by spontaneous self-pollination and cross-pollination reinforces the hypothesis that stylar movement in *Talinum fruticosum* can facilitate or hinder self-pollination in some situations.

Keywords: floral movement; herkogamy; pollination; Pantanal

During the course of angiosperm evolution, flowers underwent morphological changes to reduce the interference between male and female functions in hermaphroditic plants, including: 1) spatial separation between male and female reproductive organs (herkogamy), 2) temporal separation of sexual functions (dichogamy), and 3) reduction of male-female interference through the movement of floral structures (movement herkogamy) (Ruan & Silva 2011, Cardoso *et al.* 2018). The ability of plants to reproduce autonomously increases the species changes to colonize new environments, and to expand under conditions of low density or pollen and pollinator limitation (Randle *et al.* 2009). Herkogamy in

autonomously self-pollinating of flowers can increase species fitness but some species avoid this strategy by reducing floral maturation period and size (Snell & Aarssen 2005) or through floral movements such as stigma curvature (Klips & Snow 2018).

Floral movements can promote self-pollination as a mechanism of reproductive assurance (Ruan & Silva 2011). Delayed self-pollination occurs at the end of anthesis through coiling of the stigma branches that come in contact with the stamen (Rathcke & Real 1993). In this case, self-pollination would be facultative, and movement of floral parts only occurs in the absence of prior vector-mediated pollination.

Talinum fruticosum (L.) Juss. (Talinaceae) is commonly known as 'breço', purslane (Agra *et al.* 2008), and water leaf (Aja *et al.* 2010). The species has large populations and is used in the native cuisine and for medicinal purposes to treat or prevent diseases, increasing immunity due to its immunostimulatory activity (Brasileiro *et al.* 2010). In Brazil, this species grows spontaneously or subspontaneously, forming subpopulations in open and ruderal areas.

In this short communication, we report the results of stilar movement used by a native species of Brazil to ensure its reproductive success. We also determine multiple floral measurements: flower opening time, pollen viability and the period that the stigma was receptive. Mating system was described, and we showed how stilar movement is related to species reproductive strategy. For this, two natural populations of *T. fruticosum* located in the Central-West region of Brazil were observed from August to October 2016 and from January to March 2017. One population was located in Cuiabá-MT (15° 56' 49" S and 56° 6' 16" W) and the other in Corumbá-MS (19° 34' 42" S and 57° 0' 37" W). The distance between the two populations studied was about 1,100 km. Each *T. fruticosum* population consisted of 50-80 individuals at anthesis stage.

Firstly, pollen viability was determined during anthesis of *T. fruticosum* flowers. Five packed anthers of different flowers collected from ten individuals of each subpopulation were evaluated. The anthers were removed and crushed on glass slides and 3% carmine dye was applied (Kearns & Inouye 1993). Pollen viability was determined by counting 200 pollen grains per slide. Stigma receptivity was evaluated in the same flowers as used for the assessment of pollen viability by reaction with 3% hydrogen peroxide (Kearns & Inouye 1993).

To understand the process of the stilar movement in *T. fruticosum* and to determine the stage of anthesis when flowers perform self-pollination, ten subpopulations (with 05 to 10 individuals) of each population were observed. The flower buds of different individuals from the natural subpopulations were monitored throughout floral anthesis. During the observations, floral measurements such as the flower opening process, the position of stamens

and pistil, the duration of each phase, and the movement of stigma were recorded. The phases were classified according to flower opening, opening of stigma lobes, and stilar movement.

To determine the predominant mating system in *T. fruticosum*, 20 individuals originating from the two populations, randomly collected, were transplanted into 18 x 25 cm pots and kept in a greenhouse. Spontaneous self-pollination, hand cross-pollination, apomixis, and natural pollination treatments were performed. The occurrence of spontaneous self-pollination was evaluated in previously packed in ten flowers of each population. Hand cross-pollination was carried out between individuals of the two populations, in which each population ten flowers was a pollen donor and the other, ten flowers, a recipient and vice versa. Apomixis was evaluated by removing the stamens of the ten flowers, from each population, previously packed in the pre-anthesis and natural pollination was recorded by placing the twenty pots outside the greenhouse and exposing the flowers to floral visitors.

The flowers of *T. fruticosum* were composed of 2 sepals and 5 petals of pink to lilac colors, ranging from 7.14 -10 mm in length, actinomorphic symmetry. The number of stamens varies from 11 to 35 among flowers of the two populations. The androecium consisted of six sets of stamens, three sets of larger stamens that ranged in length from 3.1 to 4.2 mm in the Cuiabá population and from 4.0 to 5.4 mm in the Corumbá population, and three sets of smaller stamens ranging from 2.5 to 3.0 mm in the Cuiabá population and from 2.8 to 3.8 mm in the Corumbá population. The pistil consisted of trifid stigma whose heights ranged from 5.6 to 7.9 mm when erect (Figure 1A) and from 3.6 to 4.8 mm when curved in the populations (Figure 1B). Herkogamy ranged from 0.7 to 1.3 mm in the Corumbá population and from 1.4 to 2.1 mm in the Cuiabá population.

The flowers of *T. fruticosum* opened between 4:30 to 5:00 a.m. Petals started to wilt at 10:30 a.m., and this process was finished by 11:30 a.m. of the same day. Pollen viability ranged from 65 to 94% at the populations evaluated (Figure 2). Our observations also showed that stigmas began to become receptive at 9 a.m. and were receptive in 100% of the flowers until the end of the floral anthesis, around 11:30 a.m.

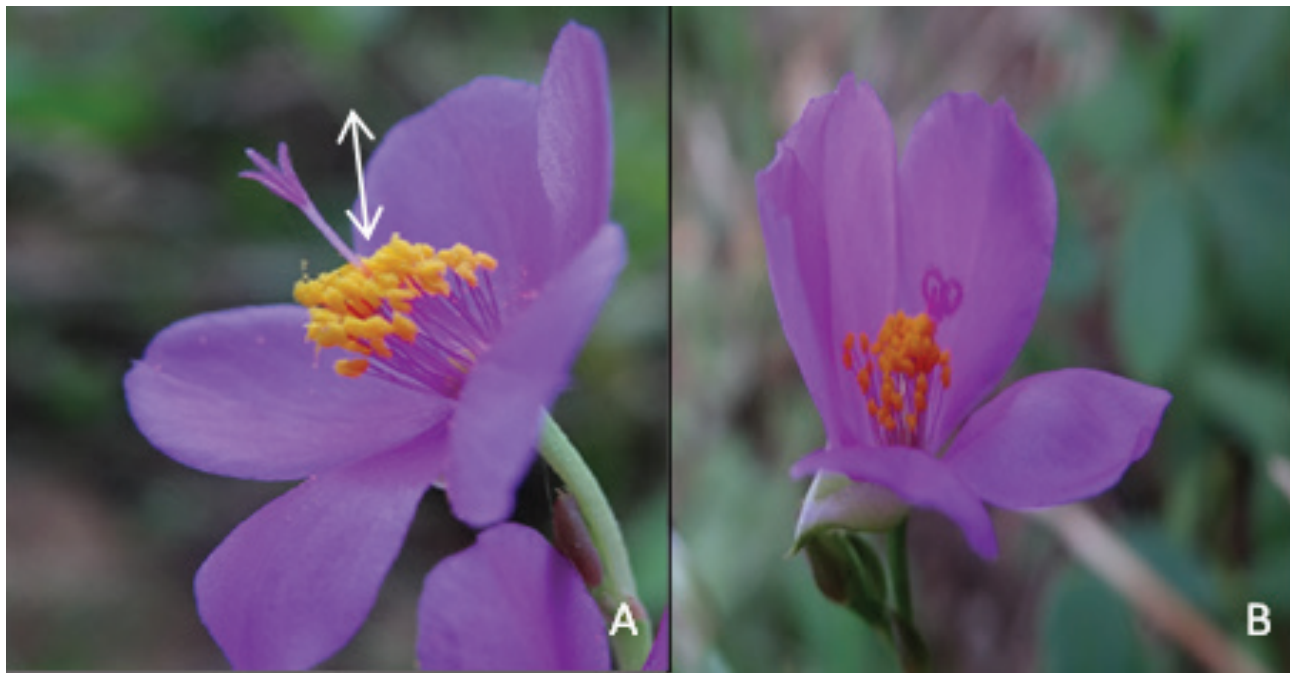


Figure 1. *Talinum fruticosum* flower. Erect stigma (A) and curved (B).

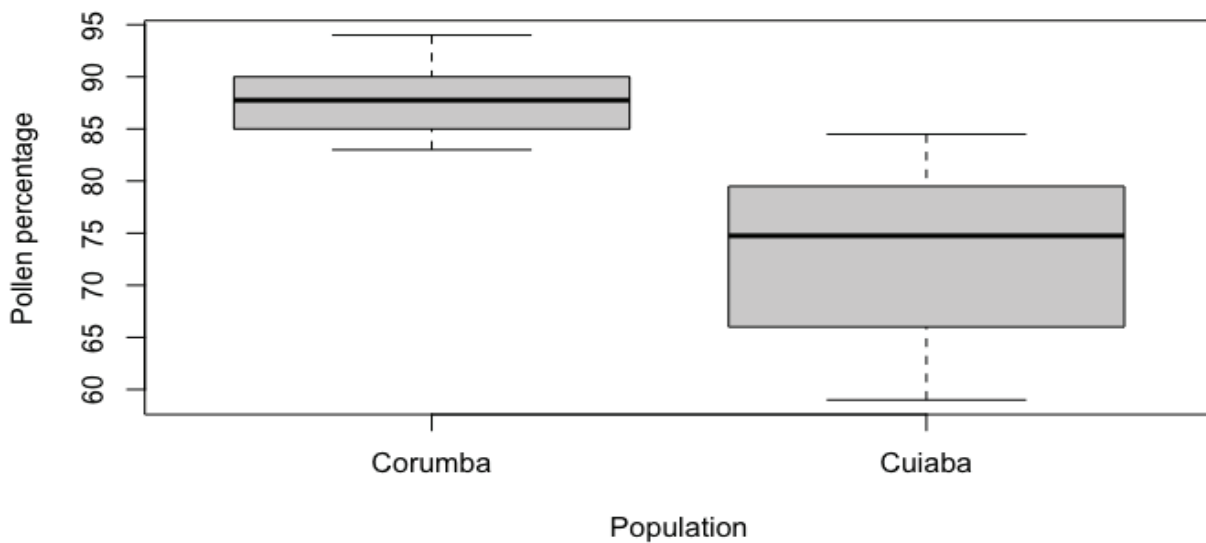


Figure 2. Pollen viability of the populations of *Talinum fruticosum* analysed.

Stylar movement of *T. fruticosum* can be subdivided into four different stages:

Stage I: Beginning of anthesis. At this stage, the petals are fully extended, the anthers are opened and releasing pollen, and the stigma is positioned above the anthers with the stigma branches closed, indicating the male phase. The male phase (protandry) lasts about one hour after flower opening (Figure 3, phase I).

Stage II: Opening of stigma branches. At this stage, the stigma branches are positioned above the anthers. This stage characterizes the end of

protandry and the beginning of the decline in herkogamy between the pistil and stamens due to the coiling of stigma branches (Figure 3, phase II).

Stage III: This stage characterizes the beginning of lateral style movement (Figure 3, phase III).

Stage IV: This stage characterizes the lateral positioning of the style between petals (Figure 3, phase IV).

To understand the stylar movement described above it is necessary to comprehend the reproductive system of the species. In the two populations evaluated during the study, the

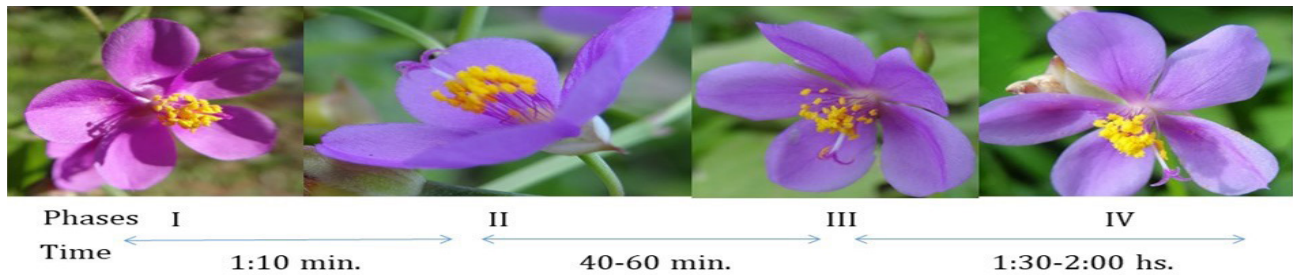


Figure 3. Stages of stilar movement in *Talinum fruticosum* flowers. Phase I: Complete distension of the petals, anthers releasing pollen, and united stigma branches positioned above the anthers. Phase II: Opening of stigma branches. Phase III: Increase in stigma curvature or lateral movement of the style. Phase IV: Positioning of the style between petals.

flowers of *T. fruticosum* produced 100% fruit by spontaneous self-pollination. Cross-pollination and natural treatments the percentage of formed fruits was from 80 to 100%. No formation of fruits was observed in the apomixis treatment. In the spontaneous self-pollination experiment, the first contact of the stigma with pollen occurred within the first three hours after anthesis and progressed until the end of anthesis. About 2:00 to 2:45 hours after the beginning of floral anthesis, the stigma branches were completely curved (Figure 4).

No curvature of the stigma branches was observed in the hand cross-pollination treatment. However, a lateral movement of the style until it touches the corolla was observed.

Our observations showed that two situations can occur in the natural pollination experiment. If there are no visits from pollinators, about two to three hours after the beginning of anthesis, the stigma begins to curve and will touch the anthers promoting self-pollination. However, in the case of a visit from natural pollinators, the style moves laterally until it touches the corolla, or it is positioned between the petals, maintaining herkogamy. Late self-pollination is considered an adaptive trait because it apparently ensures seed production when pollinators are scarce but allows

vector-mediated cross-pollination to take place when possible (Snell & Aarssen 2005).

In this study, a short period of opening flower was observed for the two populations of *T. fruticosum* evaluated. Nevertheless, short periods of opening flower were also observed for other species of this botany family (Pinto & Schindwein 2015), although in the current study levels of pollen viability were much lower than the study cited above.

Previous studies observed a lack of correlation between herkogamy and outcrossing rate (Elle & Hare 2002). However, researchers had found that herkogamy can prevent self-fertilization but in times of absence of pollinators coexistence of self-pollination and herkogamy and increase the reproductive success of species (Herlihy & Eckert 2007). Furthermore, stigma curvature resulting in contact between the distal portion of the stigma and the pollen itself also has been reported in the botany family Malvaceae (Klips & Snow 1997, Ruan *et al.* 2010). Stigma curvature has been proposed as a mechanism of reproductive assurance of species in the absence of pollinators (Shetler 1979). In this study, the stilar movement of the *T. fruticosum* in response to a simulated cross-pollination visit increases the reproductive assurance and maintenance of genetic variability. Solitary bees are

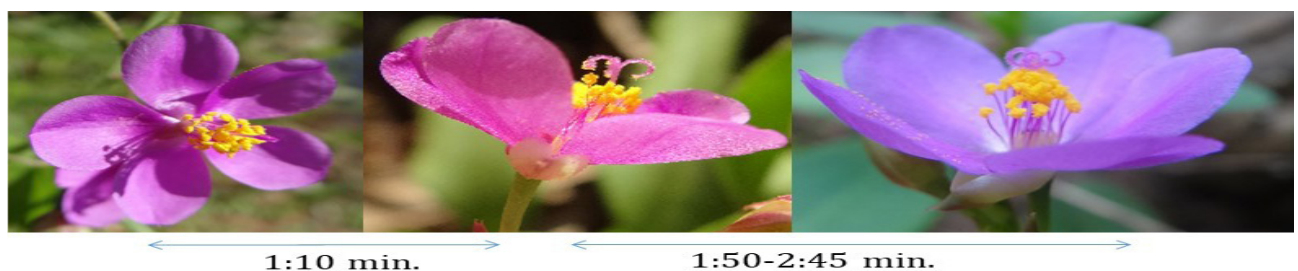


Figure 4. Stilar movement during self-pollination.

some of the most frequent visitors of *T. fruticosum* flowers and when feeding on pollen, these pollen collectors can deplete the pollen available for selfing.

Self-pollination was observed in our study as an alternative strategy of the species to guarantee fertilization of the opening flowers. The presence of floral mechanisms that facilitate self-fertilization is common among plants with less specialized pollination systems (Fenster & Martén-Rodrigues 2007). In general, the evolution of self-fertilization in plants has been interpreted as a mechanism to ensure reproductive success whenever pollinator visits are scarce or the number of potential mates in the population is small (Charlesworth 2006). For example, in epiphytic plants, autogamy represents a mechanism to compensate for the apparent reduced capacity of these plants to attract pollinators due to their small floral display and highly aggregated spatial distribution in the forest canopy (Bush & Beach 1995). On the other hand, self-fertilization may increase inbreeding due to the apparent reduction of seed production from hand self-pollinated flowers compared to cross-pollination, suggesting a decrease in female fitness (Lloyd 1992).

In this short communication on floral measurements like, time of opening flower, pollen viability and receptivity of the stigma were reported for *T. fruticosum*. However, our main conclusion from this study was to demonstrate how *T. fruticosum* promotes self-pollination to facilitate fertilization in the absence of pollination and how stylar movement is important to facilitate cross-pollination of this plant species. This complex reproductive system has never been reported before for this species, but it seems to be a successful approach for the survival of *T. fruticosum* in populations of Central-West Brazil.

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