

Floral biology of four *Ipomoea* (Tubiflorae: Convolvulaceae) species

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ABSTRACT. This research was carried out to evaluate the floral biology of *Ipomoea acuminata*, *I. batata*, *I. cairica* and *I. quamoclit* in Maringá, state of Paraná. Anthesis period, stigma receptivity, viability of the pollen grains and medium height of the style and filament were analyzed during the blossom period of *Ipomoea*. Both *I. quamoclit* and *I. potato* had their flowers opening at 6:00 a.m. and their closing at 2:00 p.m. The test of stigma receptivity demonstrated that *I. quamoclit* was receptive between 6:00 a.m. and 9:00 a.m. and *I. batata* between 6:35 a.m. and 1:00 p.m. The period of higher viability of the pollen grains in *I. quamoclit* was the same of the stigma receptivity. In that species, there was a reduction in the frequency of viable grains of pollen next to the time of the flowers closing. There were highest frequencies of viable pollen grains in *I. batata* between 6:35 a.m. and 7:00 a.m.; the variations in the frequency of viability of the pollen grains keep along the day until the flowers closing. In *I. acuminata*, the medium height of the style was 27.245 ± 2.431 mm and the medium height of the filament was 26.439 ± 3.147 mm. In *I. batata*, the medium height of the style was $19.77 \text{ mm} \pm 1.956$ mm that is higher than the medium height of the filament. The medium height of the style in *I. cairica* was 19.827 mm and the medium height of the filament was 17.952 mm. In *I. quamoclit* the medium height of the style and the medium height of the filament had both bigger and smaller height variations of the style. For that species the estimated differences between the medium height of the style at 8:30 a.m. and the filament 1 and 4; and between the medium height of the style at 10:30 a.m. and filament 3, 4 and 5 were significant. For *I. batata* the differences between the medium heights of the style and of the filament were significant.

Key words: floral biology, *Ipomoea*, pollen, anthesis period.

RESUMO. Biologia floral em quatro espécies de *Ipomoea* (Tubiflorae: Convolvulaceae). O objetivo deste trabalho foi estudar a biologia floral de *Ipomoea acuminata*, *I. batata*, *I. cairica* e *I. quamoclit* na região de Maringá, Estado do Paraná. Durante o período de floração de *Ipomoea* foram avaliados: período de antese, receptividade do estigma, viabilidade dos grãos de pólen e altura média do estilete e dos filetes. Tanto *I. quamoclit* quanto *I. batata* apresentaram abertura das flores às 6h e fechamento completo às 14h. O teste de receptividade do estigma demonstrou que em *I. quamoclit* estava receptivo entre 6h e 9h e *I. batata* entre 6h35min e 13h. O período de maior viabilidade dos grãos de pólen em *I. quamoclit* coincide com o de receptividade do estigma. Nessa espécie, próximo ao horário de fechamento das flores ocorreu diminuição na frequência de grãos de pólen viáveis. As maiores frequências de grãos de pólen viáveis em *I. batata* ocorreram entre 6h35min e 7h, as variações na frequência de viabilidade dos grãos de pólen se mantêm ao longo do dia até o fechamento das flores. Em *I. acuminata* a altura média do estilete foi de 27,245 mm ($\pm 2,431$) e a altura média dos filetes foi de 26,439 mm ($\pm 3,147$). Em *I. batata* a altura média do estilete foi de 19,77 mm; $\pm 1,956$ mm que é maior que a altura média dos filetes. A altura média do estilete em *I. cairica* foi de 19,827 mm e a altura média dos filetes foi de 17,952 mm. Em *I. quamoclit* a altura média do estilete em relação à altura média dos filetes variou desde aqueles abaixo até aqueles acima do estilete. Para essa espécie as diferenças estimadas entre a altura média do estilete às 8h30min e os filetes 1 e 4; e entre altura média do estilete às 10h30min e filetes 3, 4 e 5 foram significativas. Para *I. batata* as diferenças encontradas entre as alturas médias do estilete e dos filetes foram significativas.

Palavras-chave: biologia floral, *Ipomoea*, pólen, período de antese.

Introduction

The *Convolvulaceae* family presents wide distribution in tropical and subtropical areas. They are, in general, plant creepers, but they can also occur as bushes and small trees (Joly, 1994). Their species are classified in several categories as weed (Aranha *et al.*, 1988; Lorenzi, 1991), medicinal plants, and it can also be used for human feeding.

Also, they can eventually be used to beautify fences and wires in gardens because of its good-looking and colored flowers in bell form (Proctor and Yeo, 1972).

Palinologic studies reveal that this family shows registrations starting from approximately 65 million years ago (Roubik, 1992). These data are important to relate the evolution, in geologic time, of the plants with its respective pollinator agents.

Countless species of several weed families are found in Maringá because of its agricultural area. Among them, the "viola rope": *I. cairica*, *I. aristolochiaefolia*, *I. purpurea* and *I. quamoclit*.

The study of reproduction forms and pollination mechanisms of weed are essential because they provide important information for the elaboration of control programs (Blanco, 1972).

It is important to know the competitive ability of weed as it is a basic element for the integrated handling. Knowing that will allow to foresee the losses of income caused by them and also, what kind of practice can avoid these losses. (Oliver *et al.*, 1976; Shurtleff and Coble, 1985).

Rizzardi *et al.* (2004) verified that the incidence and the density of the "viola rope" in soybean crop increased in the last years, thus, reducing the productivity and harming the harvest.

The "viola rope" is an aggressive competitor that benefits itself from the presence of another weed in soybean crop. Rizzardi *et al.* (2004) verified that when there is 100% of "viola rope" in the soybean crop, the losses were of 54%. When there is "viola rope" only in *euphorbia heterophylla*, the losses are reduced to 25%.

In that way, this research was carried out to evaluate some aspects of the floral biology of *Ipomoea acuminata*, *I. potato*, *I. quamoclit* and *I. cairica* distributed in the Campus of State University of Maringá – UEM (*Universidade Estadual de Maringá*).

Material and methods

Plants from the family *Convolvulaceae* of *I. acuminata*, *I. potato*, *I. cairica* and *I. quamoclit*, in the Campus of State University of Maringá (UEM) were used. Maringá is in the northwest area of state of

Paraná at 554 m of altitude and 23° 24' of south latitude and 51° 25' of longitude west with humid subtropical climate (type Cfa). The annual average temperature is 21.9°C and annual pluviometric precipitation of 1,500 mm with influence of cold fronts of the south area.

The flowers and fruits development were accompanied during one year. The flower buds were marked at random with numbered labels. These buds were accompanied through periodic observations, since the opening until the flowers withering and that time was considered like anthesis period.

The stigma receptivity to the pollen grains was evaluated in five flowers collected every two hours (from 7:00 a.m. to 5:00 p.m.), during the flowering. The presence of peroxidase enzyme reflects the stigma receptivity. The stigma was immersed into hydrogen peroxide (3%) and the bubbles were observed (Dafni, 1992).

In order to verify the pollen grains viability in the flowering period, five flowers from each treatment were collected every two hours (from 7:00 a.m. to 3:00 p.m.). Five hundred pollen grains were analyzed in each lamina, following the Radford *et al.* (1974) technique. For each species, the average height of the style and the filament of each flower was measured.

The averages were compared, using the Tukey test using GLM of SAS (Sas Institute, 1998).

Results and discussion

Ipomoea acuminata

In Table 1, data show that the medium height of the style of the *Ipomoea acuminata* flowers was 27.245 ± 2.431 mm of a total of 53 measured flowers, while the medium height of the filament was 26.439 ± 3.147 mm. However, the medium height of the filament 4 and 5 (Table 1) is larger than the medium height of the style. In general, those results suggest that it should have a small incidence of self-pollination.

Table 1. Medium height (mm), standard deviation and number of flower of *Ipomoea acuminata* analyzed by morphometry.

	Medium height (mm)	Standard deviation	Number of observations
style	27.245	2.431	53
Filament 1	24.380	2.738	53
Filament 2	25.745	2.776	53
Filament 3	26.717	2.097	53
Filament 4	27.585	2.663	53
Filament 5	28.176	3.598	51
Average of five filament	26.439	3.147	

In Figure 1, data show that the differences

between the height of the style and the filament were significant for Filament 1. In that case the filament was inferior to the style, suggesting that, in general, there are mechanisms that prevent the self-pollination.

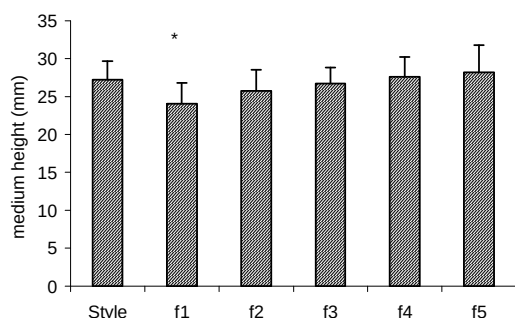


Figure 1. Test of multiple comparison of Tuckey-Kramer among the medium of height of filament and style for *Ipomoea acuminata*. (* = significative values - $p < 0.05$). f1 = Filament 1; f2 = Filament 2; f3 = Filament 3; f4 = Filament 4; f5 = Filament 5.

Ipomoea potato

In *Ipomoea potato* the medium height of the style was $19,773 \pm 1,956$ mm that is higher than the average of the height of the filament (Table 2). Those results were confirmed with the test of comparison of averages between the medium height of the style and the filament. It demonstrates that the estimated differences are significant (Figure 2).

Table 2. Medium height (mm), standard deviation and number of flower of *Ipomoea batata* analyzed by morphometry.

	Medium height (mm)	Standard deviation	Number of observations
Style	19,773	1,956	22
Filament 1	15,227	2,671	22
Filament 2	15,341	1,728	22
Filament 3	16,045	2,126	22
Filament 4	16,295	2,515	22
Filament 5	15,333	2,517	21
Average of five filament	15,651	2,333	

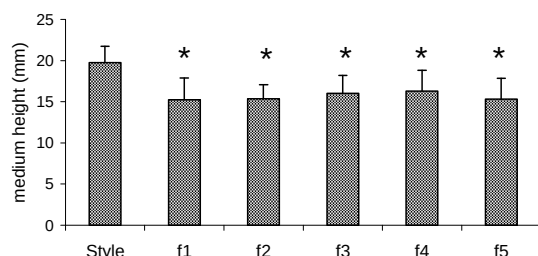


Figure 2. Test of multiple comparison of Tuckey-Kramer among the medium of height of filament and style for *Ipomoea batata*. (* = significative values - $p < 0.05$). f1 = Filament 1; f2 = Filament 2; f3 = Filament 3; f4 = Filament 4; f5 = Filament 5.

Results showed that the flowers opening of *I.*

potato happens at 6:00 a.m. and the closing at 2:00 p.m.

At 7:00 a.m. *Apis mellifera* was observed visiting the flowers.

The test of receptivity of the stigma showed that this was receptive between 6:35 a.m. and 1:00 p.m.

In Table 3, data show that the highest frequencies of viable pollen grains are between 6:35 a.m. and 7:00 a.m. Those results demonstrate that there are variations in the frequency of viability of the pollen grains, but this remains along the day until the flowers closing (Figure 3A).

Table 3. Viability of pollen grains during the day in *Ipomoea batata*.

Horary	Number of pollen grains			Frequency of pollen grains	
	Viable	Unviable	Total	Viable	Unviable
06:35	290	4	294	0,987	0,013
07:00	234	6	240	0,975	0,025
08:00	448	56	504	0,889	0,111
09:00	425	32	457	0,93	0,07
10:00	223	52	275	0,811	0,189
11:00	29	3	32	0,91	0,09
12:00	73	13	86	0,849	0,151
13:00	25	5	30	0,834	0,166
Total	1747	171	1918	0,911	0,089

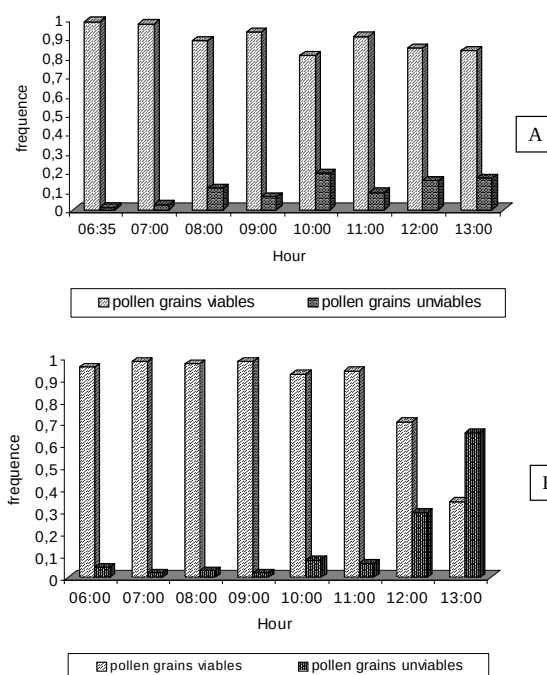


Figure 3. Viability of pollen grains of *Ipomoea batata* (A) and *Ipomoea quamoclit* (B) during the day.

Ipomoea cairica

For *Ipomoea cairica*, collections of flowers were accomplished in three schedules along the day. Results showed that in Table 4 only the medium height of the Filament 5 at 8:00 a.m. was higher than medium height of the style in that time.

Table 4. Medium height (mm), standard deviation and number of flower of *Ipomoea cairica* analyzed by morphometry in three times during the day.

	Medium high (mm)	Standard deviation	Number of observations
Style - 8 h	20.000	0.471	10
Filament 1	16.200	1.636	10
Filament 2	19.150	3.440	10
Filament 3	18.300	3.302	10
Filament 4	17.700	3.368	10
Filament 5	21.556	3.005	9
average of five filament	18.520	3.388	
Style - 9 h	19.763	0.788	19
Filament 1	18.868	3.979	19
Filament 2	18.053	2.723	19
Filament 3	19.526	3.356	19
Filament 4	18.763	3.773	19
Filament 5	18.579	3.481	19
average of five filament	18.758	3.447	
Style - 11 h	19.717	1.808	30
Filament 1	16.367	3.088	30
Filament 2	17.100	3.172	30
Filament 3	17.467	3.383	30
Filament 4	18.117	2.935	30
Filament 5	18.981	3.298	27
average of five filament	17.578	3.254	

These results suggest that in *I. cairica* the self-pollination could occur. According to Galletto and Bernardello (2004) this species are self-incompatible.

In Figure 4, data show that the estimated differences were only significant between the medium height of the style and filament 1 at 11:00 a.m.

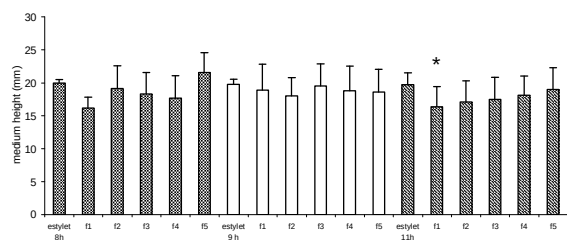


Figure 4. Test of multiple comparison of Tuckey-Kramer among the medium of height of filament and style for *Ipomoea cairica* in three hours during the day (* = significative values - $p < 0.05$) f1 = Filament 1; f2 = Filament 2; f3 = Filament 3; f4 = Filament 4; f5 = Filament 5.

Ipomoea quamoclit

In Table 5, data show that in two analyzed times of *Ipomoea quamoclit*, the medium height of the style in relation to the medium height of the filament have both bigger and smaller height variations of the style.

In Figure 5, data show that the average differences were significant between the medium height of the style at 8:30 a.m. in filaments 1 and 4; and between the medium height of the style at 10:30 a.m. in filaments 3, 4 and 5.

Table 5. Averages, standard deviation and number of flower of *Ipomoea quamoclit* analyzed by morphometry in two times during the day.

	Medium high (mm)	Standard deviation	Number of observations
Style - 8:30	30.000	1.000	45
Filament 1	28.556	1.198	45
Filament 2	29.311	1.184	45
Filament 3	29.889	1.153	45
Filament 4	30.533	1.179	45
Filament 5	31.000	1.091	43
Average of five filament	29.847	1.441	
Style - 10:30	28.841	1.357	69
Filament 1	28.594	1.386	69
Filament 2	29.101	1.226	69
Filament 3	29.638	1.188	69
Filament 4	30.159	1.290	69
Filament 5	30.559	1.286	68
Average of five filament	29.607	1.453	

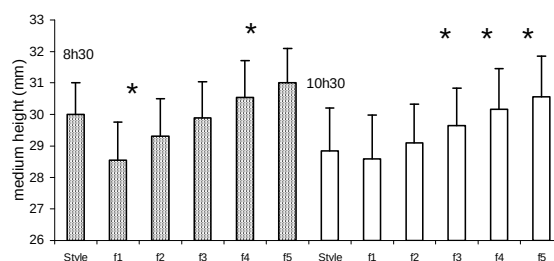


Figure 5. Test of multiple comparison of Tuckey-Kramer among the medium of height of filament and style for *Ipomoea quamoclit* in two hours during the day (* = significative values - $p < 0.05$) f1 = Filament 1; f2 = Filament 2; f3 = Filament 3; f4 = Filament 4; f5 = Filament 5.

The period of higher stigma receptivity was observed between 6:00 a.m. and 9:00 a.m.

In Table 6, results show that the period of higher viability of the pollen grains coincides with the one of stigma receptivity that is between 6:00 a.m. and 9:00 a.m. It is possible to observe in Figure 3B that along the day there are small variations in the viability of the pollen grains.

Table 6. Viability of pollen grains during the day in *Ipomoea quamoclit*.

Hour	Number of pollen grains			Frequency of pollen grains	
	Viable	Unviable	Total	Viable	Unviable
06:00	288	13	301	0.957	0.043
07:00	485	10	495	0.980	0.020
08:00	103	3	106	0.971	0.029
09:00	238	5	243	0.980	0.020
10:00	106	9	115	0.922	0.078
11:00	181	12	193	0.938	0.062
12:00	36	15	51	0.706	0.294
13:00	11	21	32	0.344	0.656
Total	1160	75	1235		

It is interesting to point out that the frequency of viable pollen grains decrease mainly at 1:00 p.m. next to the time of the flowers closing (12:00 a.m., 1:00 p.m.) (Figure 3B).

The obtained results are similar to those found by Maimoni-Rodella and Rodella (1992), studying the floral biology of *Ipomoea acuminata*. Those authors verified that the flowers of that harmful species of *Ipomoea* also have anthesis diurnal period and they are ephemeral. However, the flowers open between 4:30 a.m. and 5:30 a.m., presenting the exposed pollen grains and the receptive stigma; the withering happens between 10:00 a.m. and 12:00 a.m. In that same work, the authors verified that when withering, the corolla suffers an enrollment towards the center of the flower, obstructing the entrance of the floral tube, coming off the following morning.

However, in *Ipomoea acuminata* the most frequent visitors are bees, but some Lepidoptera and Coleoptera are also observed. Regarding to Hymenoptera species of the families, Andrenidae, Anthophoridae, Apidae and Halictidae were registered. The most abundant and efficient species in the pollination belong to the family Anthophoridae; standing out *Melitoma segmentaria* (Maimoni-Rodella and Rodella, 1992). In that species the formation of 14 fruits was observed with normal and healthy seeds, happening therefore self-pollination in 56% of the tested flowers (Maimoni-Rodella and Rodella, 1992). These data suggest that the species is autogamic, benefiting also the visit of insects that promote certain proportion of cross-pollination. This characteristic group is very frequent to the family of Convolvulaceae (Stucky, 1984; Maimoni-Rodella and Rodella, 1986, 1986/87; Machado and Sazima, 1987).

The *Ipomoea* is weed and highly competitive plants with other weed species (Rizzardi *et al.*, 2004). In this way, it would be interesting that these plants presented crossed breeding by means of pollination. This would lead to an ample genetic variability, contributing with its permanence in some types of environments. Morphometric data suggest that 4 analyzed species of *Ipomoea* have crossed breeding, probably pollinated by insects.

Galetto and Bernardello (2004) evaluated the floral morphology and the composition of the nectar in 6 species of *Ipomoea*. The authors verified that there is not a correlation between these characteristics and the pollinators of these species. Nevertheless, nectar production dynamics and removal effects, with sugar composition, could not be clearly related either to the pollinator guild or to the breeding system of the species involved. The self-incompatible species are all bee-pollinated (*I. hieronymi*, *I. indica*, *I. cairica*) and they showed a similar total nectar production, but had differences

in their nectar composition (*I. hieronymi* and *I. indica* are sucrose-dominant, whereas *I. cairica* is hexose-dominant). The self-compatible species (*I. alba*, *I. purpurea*, *I. rubriflora*) showed no variation in nectar composition (sucrose-dominant nectars), but significant differences in their nectar production pattern. In contrast, flower length was associated with both nectar size and total amount of nectar produced. Recent studies suggest that flower morphology is evolutionarily more labile and that corolla traits can frequently change (Cubas *et al.*, 1999; Harrison *et al.*, 1999) in comparison to changes in the nectar features (Galetto *et al.*, 1998; Perret *et al.*, 2001; Torres and Galetto, 2002). The association found here between flower size and total nectar volume secreted in *Ipomoea* suggests that structural constraints may play a major role in conserving nectar traits, at least in volume.

Schondube and del Rio (2002) studied the evolution and the correlation between the form of bill birds, the form of corolla, the nectar collection, and its importance in the pollination *Ipomoea* for *Diglossa*. The authors observed experimental evidence of a trade-off associated with the evolution of a morphological novelty. Moreover, they indicate that a slight hook might have provided ancestral flower piercers with increased efficiency to pierce and extract nectar from hummingbird-pollinated flowers with sympetalous tubular corollas. Subsequent increases in hook length would have provided flower piercers further access to a diverse range of floral nectar sources unavailable to legitimate, non-robbing, floral visitors. However, a longer hook that allows more effective robbing entails a cost: flower piercers with longer hooks are less efficient frugivores. The differences in hook length among flower piercers with its concomitant trade-off and contrasting diets are also associated with broad interspecific differences in aggressive behaviour and habitat use (Isler and Isler, 1999), and presumably have enabled flower piercer species with and without hooked bills to coexist with each other and with hummingbirds at several Andean locations (Moynihan, 1979; Snow and Snow, 1980).

Durbin *et al.* (2003) used the flower color of *Ipomoea* as a model for an analysis of flowers evolution and adaptation in relation to mutations in regulation genes expression. Changes in regulatory genes, on the other hand, often result in novel patterns of pigmentation that can influence pollinator behavior or even attract other types of pollinators. For instance, if natural pollinators decreased in the environment of a species, a change in pigment patterns could attract other types of

pollinators ensuring pollination, a presumably beneficial event. Also, a change in pigment patterns could lead to genetic isolation due to a change in pollinator preference. Genetic isolation could ultimately lead to the evolution of a new species. In addition to the large impact changes in expression patterns can have, there are many more ways in which mutations in regulation can occur when many different types of genes involved in regulation and the seemingly redundant tissue specific regulatory genes are considered. Small changes in regulation of the flavonoid pathway can effect large changes in phenotype and adaptive success. Although the molecular basis for the changes is not known, the regulation of expression of genes of the flavonoid pathway in the species is observed in this study. It appears that changes in regulation of gene expression are the main driving force behind the adaptive and phenotypic evolution of flower color in *Ipomoea*.

Ipomoea is a weed that presents wide diversity in ways for its reproduction and competition with other weed. However, some aspects of the pollination and pollinators need to be understood for the development of control programs of these plants.

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