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BREEDING SYSTEM AND RELATED FLORAL FEATURES UNDER NATURAL AND EXPERIMENTAL CONDITIONS OF *PASSIFLORA SUBEROSA* (PASSIFLORACEAE)

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Summary: The breeding system, the floral characteristics and the visitors of the polymorphic *Passiflora suberosa* were studied in different natural populations of Corrientes and Misiones provinces, and at a partially-opened greenhouse in Buenos Aires. The different reproductive indexes calculated evidenced that, although it is highly self-compatible, autogamy is partial. The reproductive efficiency was similar in the field and in the greenhouse. The major relative reproductive success obtained by induced self-pollination suggests that the reproductive output may be improved with the help of pollinations, either natural or artificial. Although the single flower and the blooming are inconspicuous, the floral characters may be involved in wasps attraction. The scarcity or even absence of pollinators (depending on the sites) seems to be compensated with the capacity of autogamy and the extended flowering period. The successful treatments performed in the greenhouse and the long period of time the fruits remain on the plants constitute stimulant features to cultivate this ornamental, medicinal, edible and tinctoreous species.

Key words: *Passiflora suberosa*, breeding system, self-compatibility, floral morphology.

Resumen: Sistema reproductivo y caracteres florales relacionados en condiciones naturales y experimentales de *Passiflora suberosa* (Passifloraceae). El sistema reproductivo, las características florales y los visitantes de la polimórfica *P. suberosa* fueron estudiados en distintas poblaciones naturales de las provincias de Corrientes y Misiones y en un invernáculo parcialmente abierto en Buenos Aires. Los distintos índices reproductivos calculados evidenciaron que, a pesar de que es altamente autocompatible, la autogamia es parcial. La eficacia reproductiva fue similar en el campo y en el invernáculo. El mayor éxito reproductivo relativo obtenido por autopolinización inducida sugiere que la reproducción puede ser mejorada con la ayuda de polinizaciones, ya sean naturales o artificiales. Aunque las flores por sí solas y la floración son inconspicuas, los caracteres florales pueden estar involucrados en la atracción de avispas. La escasez o incluso la ausencia de polinizadores (dependiendo de los sitios) parecen estar compensada con la capacidad de autogamia y el período extendido de floración. Los tratamientos fructíferos llevados a cabo en el invernáculo y el largo tiempo que los frutos permanecen en las plantas constituyen aspectos estimulantes para cultivar esta especie ornamental, medicinal, comestible y tintórea.

Palabras clave: *Passiflora suberosa*, sistema reproductivo, autocompatibilidad, morfología floral.

INTRODUCTION

Passiflora suberosa L. grows from the north of South America to the centre of Argentina (Deginani, 2001). In this country, it is encountered mainly in the phytogeographic provinces of the Yungas and Paranaense, but also in Chaco and Espinal. This selective hydrophyte (Da Costa Sacco, 1980) occupies sunny or partially shaded wet environments in forest edges, forest gaps, valleys and ravines (Deginani, 2001). In contrast of its wide geographical distribution, its abundance is low (Da Costa Sacco, 1980), although it was common in the past (Killip, 1938).

This species is interesting from different viewpoints. It has medicinal properties (Toursarkissian, 1980), and the edible berries (González, 1984) have staining power (Deginani, 2001), owed to their high content of anthocyanins (Kidoy *et al.*, 1997). Due to its ornamental value, some nurseries in the surroundings of Buenos Aires reproduce it by cuttings (Deginani, 2001).

The plants bloom the whole year in Brazil (Koschnitzke & Sazima, 1997). In Argentina, it has been registered in blossom almost all year round,

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except for July and August (Pedersen, unpubl.), and it produces fruits all year round, more intensely from September to May (Deginani, 2001).

Koschnitzke & Sazima (1997) studied some aspects of the floral biology of *P. suberosa* in Brazil: flowering period and anthesis, flower diameter, nectar concentration and floral visitors. Although they explored the reproductive system performing induced self-pollination and xenogamy, they did not probe spontaneous self-pollination or natural allogamy; they counted the fruits, but not the seeds produced.

Specially for rare species, conservation *ex situ* is necessary, particularly if the taxa are known to be useful for human purposes. To perform this, the reproductive events must be studied thoroughly, both in natural habitats and under experimental conditions. Considering the utility of this species, its partially researched reproductive biology and its polymorphism (Killip, 1938), the pollinators, the floral characteristics and the three components of the reproductive system (degree of self-compatibility, agamospermy and temporal or spatial separation of reproductive cycles) enumerated by Richards (1986) were studied in several natural populations and in a greenhouse to which floral visitors had access. The aim of this work was to contribute to the knowledge of the floral biology of this low abundant species, studying its components at the southern extreme of its distribution range.

MATERIALS AND METHODS

Field study sites

Walks exceeding 40 km searching for *P. suberosa* specimens were performed in the following locations, where data were recorded:

a) Cerrillos, Salta province, 24º 55’ S, 65º 29’ W (October-November, 1994): one plant in blossom near a road side.

b) Mburucuyá National Park, Corrientes province, 28º 03’ S, 58º 13’ W (October, 1998): 10 plants, 5 of which were in blossom, occupied an area of nearly 1.400 m^2, growing at the edges and gaps of a hydrophylous forest about 30 years old, where a tung plantation had taken place before. Most of the vines were climbing over *Celtis* sp. trees approximately 3 m high, displaying the majority of their buds and leaves at the trees crowns level. They were partially shaded almost the whole day (they received full sun only from time to time). The distance to the nearest conespecific neighbor was 14 ± 6 m. The suberified stem base perimeter was 2.7 ± 0.4 cm, the maximum recorded for this species (Deginani, 2001). Few other species were flowering in that forest; on the other hand, there were abundant flowers of *Glandularia* sp., *Petunia* sp., herbaceous Leguminosae, several Compositae and Calyceraceae in the surrounding grasslands, and *Eichhornia azurea*, *Sagittaria* sp., *Hydrocotyle* sp. and other entomophilous species in the aquatic environments.

c) P. Gentilini Agrotechnic School, San José, Misiones province, 27º 46’ S, 55º 47’ W (January, 1999): 28 plants in blossom were found, most of which grew at the edges of a sugar cane culture, of a peach culture and in a pine forest understory between both cultures; the rest grew at the edges of small native forests. Those in the pine forest, even if they were partially shaded, received more light than the ones at Mburucuyá National Park, as the forest was sparser; the others were more exposed to sun, but not to full sun. In the surrounding grasslands several species of Leguminosae, *Ipomoea* sp., *Passiflora caerulea* and *Asclepias mellodora* were in blossom.

d) Teyú Cuaré Provincial Reserve and Osununú Private Reserve, San Ignacio, Misiones province, 27º 17’ S, 55º 32’ W (January, 1999): a few specimens of *P. suberosa* were encountered, which were not in blossom or had very small buds, so juvenile plants were collected to perform a reproductive essay in the greenhouse of Buenos Aires University (34º 38’ S, 58º 28’ W).

Most of the observations were carried out at Mburucuyá National Park and at P. Gentilini Agrotechnic School; only when differences among localities were encountered they are mentioned in the text.

Pollination mechanisms

They were carried out in the field (Mburucuyá National Park, P. Gentilini Agrotechnic School) and in the partially-opened greenhouse of the Facultad de Ciencias Exactas y Naturales of Buenos Aires University. In this last place, the young plants collected in the field were grown in pots under partial shade and natural photoperiod. They flowered from March to May 1999, when the assay was performed, and they continued flowering every year until 2005. To study the degree of self-compatibility, spontaneous self-pollination, natural pollination, agamospermy, induced self-pollination, induced xenogamy and natural allogamy were investigated in the greenhouse; due to logistic reasons and to the low abundance of this species, the few plants in blossom and the few flowers per plant, only the first two treatments were also carried out in the field, to test for differences between sites (field vs. greenhouse). All the buds were marked and bagged before anthesis, and bagged again after the treatment received, except for the ones of spontaneous self-pollination, which were never unbagged during the assay. Those from natural pollination and natural allogamy were unbagged during anthesis, to expose them to the visitors. Anthers were removed before anthesis from the buds used to test agamospermy, natural allogamy and induced xenogamy. Pollen was transferred manually to the stigmas of the buds used to test induced self-pollination and induced xenogamy, from the same flower and from a flower of a different plant, respectively. Various reproductive indexes taken from Ruiz Zapata & Kalin Arroyo (1978) were calculated: ISI (index of self-compatibility = fruits from induced self-pollination/ fruits from induced xenogamy), IAS (index of autogamic self-pollination = fruits from self-pollination/ fruits from induced self-pollination), RE (reproductive efficacy = % fruits from natural pollination/ % fruits from self-pollination); the same indexes were also calculated using the seeds percentage. The RRS (relative reproductive success = fruits obtained/flowers treated x seeds/ovules) (Dafni, 1992) was also computed.

Floral features

In order to test for dichogamy or hercogamy and for the xenogamic or autogamic features of the flowers the following characteristics were registered during anthesis: flower orientation, floral pieces position, pollen (energetic constituents, pollenkit, vitality, and exposition), stigma receptivity, nectar (presence, concentration, and volume), existence of nectar guides (visible and UV), odor, and floral dimensions.

Pollen. To determine the preponderance of energetic constituents, reactions with sudan IV and iode solution to detect lipids and starch, respectively, were made (Johansen, 1940). Pollenkitt (the lipidic sticky coating of pollen grains dispersed by animals) existence and abundance was registered with sudan IV. Vitality was analyzed by contrast-fluorescent microscopy (Greissl, 1989).

Stigma receptivity. Different sets of previously emasculated (to avoid pollen interference) and bagged (to avoid visitors interference) buds were cut when they reach different floral phases. A drop of hydrogen peroxide 10 % was placed on the stigmas surface (Osborn *et al.*, 1988) and the formation of bubbles (the sign of the positive reaction) was observed with a dissecting microscope.

Nectar. It was sampled at each floral stage, in flowers that had been bagged when buds. Nectar was removed with 7 µl capillaries narrowed by fire. Total percentage of sugars was measured with a temperature-compensated hand refractometer.

Coloration in the visible spectrum. It was described by comparison of the floral parts from flowers at the different stages of the anthesis with the color chart of Kelly (1965).

Reflection in the UV spectrum. It was registered with photographs taken with an 18 A Kodak filter through which only UV light (300-400 nm) passes (Kevan, 1983).

Olfactory tests. They were performed during all the floral stages. The different pieces of the flowers were put in different hermetic vials and smelled after 15 minutes.

Floral dimensions. The ones related with the available surface where visitors could land (sepals and *radii* length), the space between this surface and the reproductive whorls (anthers-*radii* distance) and the degree of the hidden reward (tube depth) were measured. The major diameter of the flowers was also measured in order to assign them to a size category.

Flower visitors

Observations *ad libitum* for 60 hr were carried out during the whole anthesis period in order to detect pollinators, their activity on the flowers, and their frequency of visits.

Meteorological conditions

Ambient temperature and relative humidity were registered with a digital termohygrometer. Temperature and relative humidity varied, respectively, between 9-28 ºC and 28-92 % in the field and between 8-33 ºC and 29-99 % in the greenhouse.

Terminology

To denote the general morphology of *Passiflora* flowers the terminology from Tillet (1988) was followed.

RESULTS

Breeding system

Each inflorescence generally bears two buds. Either one or two fruits were produced by spontaneous, induced or natural pollination in each inflorescence; the percentage of fructification was equal (33 %, n = 14 inflorescences) and all reached maturity. In the greenhouse, some fruits remained fresh for 2 months without falling (when they were cut to count the seeds). The quantity of ovules/flower of the individuals from Misiones was nearly the double compared to the ones from Corrientes (mean= 30 ± 4, n = 27, vs. 18 ± 2, n = 34; *F* = 175,341, p < 0.005). The shape of the fruits was also different: those from Misiones were umbilicate in the apex while the ones from Corrientes were cordiform. Fruits were not formed neither by agamospermy nor by natural allogamy tested at the greenhouse (Table 1). Fruit production differed significantly between pollination treatments performed at the greenhouse ($Xi^2 = 52.38$, $p < 0.005$) but it did not differ between the field vs. the greenhouse, neither by natural pollination ($Xi^2 = 1.48$, $p > 0.005$) nor by self-pollination ($Xi^2 = 0.015$, $p > 0.005$). Seed production (Table 1) did not differ significantly, neither between the successful pollination treatments at the greenhouse ($F = 2.60$; $p > 0.005$) nor between the two pollination treatments tested in different localities ($F = 0.40$, $F = 0.58$, $F = 0.67$, $p > 0.005$ for all the cases). The reproductive indexes are included in Table 2.

Floral features

Flower orientation. The floral peduncle was frequently in right angle with respect to the supporting branch; if the branches were not exactly perpendicular to the ground, an upwards curvature of the peduncle was observed, so the flowers were always erect. This orientation was already present in the buds. When the fruit began to develop, the peduncle changed the curvature and the fruits hanged.

Floral pieces position. According to the movements of the reproductive organs, 3 stages were defined. Stage 1 (Fig. 1 A): sepals perpendicular to the floral axis; *radii* recurved (they resemble the position of spider legs) (Fig. 1 B, arrow); anthers in right or acute angle with the floral axis (with or without a 90° spin relative to their filament), so the dehiscent face pointed towards the corona or outwards, respectively; stigmas above the anthers. Stage 2 (Fig. 1 B): at least one stigma at the same level or below the anthers. Stage 3 (Fig. 1 C): the styles, and consequently the stigmas, went gradually upwards until they became parallel to the floral axis; the stamens bent; the *radii* and sepals incurved, closing the flower in this way.

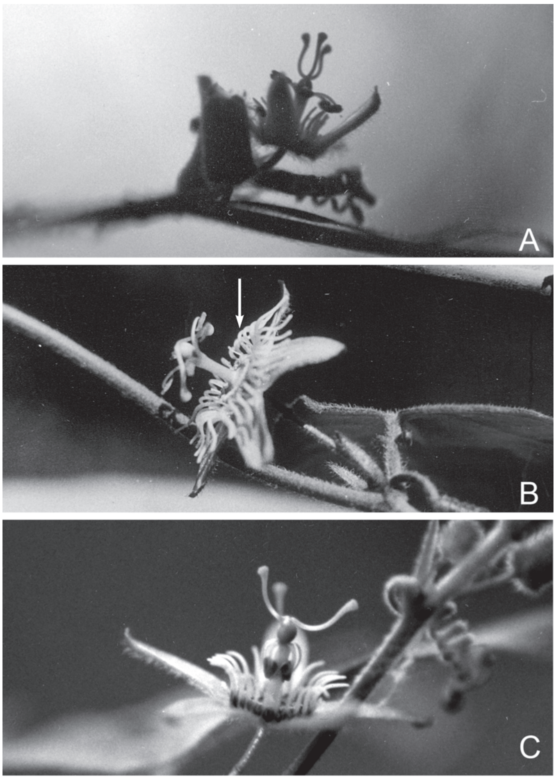


Fig. 1. Floral stages. **A:** stage 1. **B:** stage 2. **C:** stage 3. The arrow points the recurved *radii*.

Pollen

Exposition**.** When the flowers opened, the anthers were not always dehiscent; sometimes they dehisced at the end of stage 1 or during stage 2, when humidity was lower than 67 %, temperature was higher than 17-25 °C and they began to be exposed to the sun. Besides, not all the anthers opened simultaneously.

Characteristics. Only lipids were detected as energetic constituents. Scarce pollenkitt was observed. Vitality was high: 82 % (n = 206 grains).

Spontaneous self-pollen deposition. The stigmas edges became fully covered with pollen when the anthers made the spin during flower opening or when the stigmas were situated between or below the anthers during stage 2.

Stigmatic receptivity. It was positive in all the floral stages (n = 30), even when the anthers had not

Table 1. Fruit and seed production. Fruit production by pollination mechanisms with asterisks differed significantly. **SE**= standard error; **RRS** = relative reproductive success.

Pollination mechanism	fruits percentage (treated flowers/treated plants)	average seeds/fruit ± SE (processed fruits)	RRS
Greenhouse			
natural pollination	81 (16/4)	47 ± 8 (13)	0.38
spontaneous self-pollination	63 (19/4)	49 ± 8 (12)	0.33
induced self-pollination	83 (12/4)	78 ± 7 (10)	0.66
induced xenogamy	50 (4/4)	50 ± 35 (2)	0.25
natural allogamy	0 (10/4) *	------	-----
agamospermy	0 (18/4) *	------	-----
Field			
natural pollination	64 (28/6)	57 ± 8 (13)	0.36
spontaneous self-pollination	61 (39/5)	50 ± 6 (23)	0.29

Table 2. Reproductive indexes of *P. suberosa* calculated with fruits/flowers and seeds/ovules ratios. **ISI** = index of self-incompatibility; **IAS** = index of automatic self-pollination; **RE** = reproductive efficacy; **g** = greenhouse; **f** = field.

Reproductive index	ISI	IAS	RE
calculated with fruits	1.66	0.74	1.29 (g) 1.05 (f)
calculated with seeds	1.56	0.62	0.96 (g) 1.14 (f)

yet dehisced.

Anthesis. Rarely both of the 2 buds/foliar axile opened the same day; they used to open in successive days. A mean of 2 ± 1 flowers per plant opened daily (n = 11). Anthesis began at 6 a.m., when the light reached the understory (it cleared up earlier in the grasslands) and finished at ca. 7 p.m. A shorter style with a smaller stigma which did not bend during anthesis, was observed in a few flowers. The sequence of the floral stages and the relation with the meteorological conditions are shown in Fig. 2. On sunny and hot days (27/X), the flowers reached stage 3 by 5 p.m., and were closed by 7 p.m. The change to stage 3 was a little delayed when it was cloudy and windy (24/X), and anthesis was considerably prolonged when it rained (23/X) (flowers were still in stage 3 in the morning of the third day since anthesis). Rain delayed more the anthesis than low temperature (cfr. days 23 and 24).

Nectar. It was found in the nectar chamber as soon as the flowers opened, and in all floral stages. The total percentage of sugars was 36 ± 9 (n = 8 flowers from 8 plants) during the whole anthesis. The volume was less than 7 µl in each floral stage.

Visual pattern. The coloration in the visible spectrum and the reflection in the visible and UV spectra did not vary throughout anthesis.

Coloration in the visible spectrum. Sepals (abaxial face): base dark violet, strong yellow green the rest; sepals (adaxial face): brilliant yellow green; *radii*: base dark violet, brilliant yellow green the middle; tip: light greenish yellow; *pali*: basal half dark violet, brilliant yellow green the apical half; *operculum*: tip white, violet the rest; limen: dark violet or brilliant yellow green; androginophore: dark violet the basal half, brilliant yellow green apical one (sometimes the whole androginophore was of this last color or brilliant yellow green with dark violet maculae); staminal filaments, anthers dorsum, ovary, styles and stigmas: brilliant yellow green (sometimes the basal part of the ovary was strong yellow green; at San José, all the ovary was brilliant yellowish green with white dots); pollen: vivid yellow. When the flowers closed, either they were pollinated or not, the abaxial face of the persistent sepals became dark violet, first, and deep reddish brown afterwards. Flowers from San José were predominantly yellow green, only the *pali* were violet.

Reflection and absorption in the visible and UV spectra. In the visible spectrum (Fig. 3 A), the base of the *radii*, *pali* and *operculum* formed a narrow dark circle that contrasted with the rest of the floral pieces; in the UV spectrum (Fig. 3 B), the *radii*, *pali* and *operculum* absorbed strongly UV light along their whole length, giving rise to a wider dark circle that opposed to the remaining reflecting floral parts. In this way, a pattern of contrasting concentric circles turned out (sepals vs. *radii-pali-operculum* vs. limen).

Odour. It was not perceived in any floral stage (n = 27).

Flower dimensions. They are shown in Table 3.

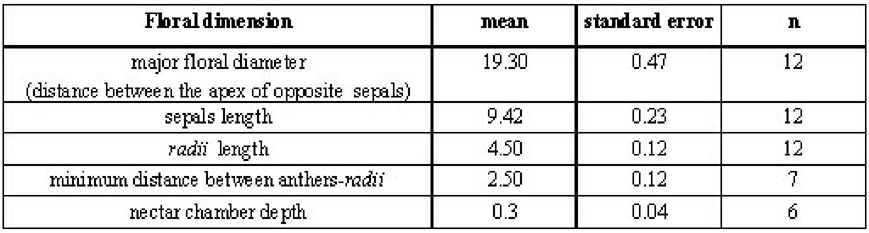
Flower visitors

Although observations were performed under different meteorological conditions and during the whole anthesis, very few visitors were observed on *P. suberosa* flowers in Mburucuyá N. P. and none in the Agrotechnic School or in the greenhouse; in spite of this, hymenopterans, lepidopterons and dipterans were active on flowers of other species that grew in the surroundings of both localities, specially on sunny and hot days, and different hymenopterans were seen flying inside the greenhouse. The activity of the visitors observed on *P. suberosa* flowers is described in Table 4.

Fruit eaters

Lepidoptera larvae and acari were found inside entire mature fruits in the greenhouse. The aril, endocarp and mesocarp had been eaten.

Table 3. Floral key dimensions (in mm) of *P. suberosa* flowers in the interaction with the visitors.



Floral dimension	mean	standard error	n
major floral diameter (distance between the apex of opposite sepals)	19.30	0.47	12
sepals length	9.42	0.23	12
radii length	4.50	0.12	12
minimum distance between anthers-*radii*	2.50	0.12	7
nectar chamber depth	0.3	0.04	6

Table 4. Floral visitors activity on the flowers at the different study sites.

Visitor	Activity	Locality
Hymenoptera		
Formicidae 2 spp.	nectar licking and stigmas contact (in stage 2) when nectar accumulated between the operculum and the elevated part of the radii	Cerrillos
Andrenidae	walks on the radii around the androginophore and pollen collection	Mburucuyá
Antophoridae *Xylocopa* sp.	exploration of only one plant of *P. suberosa*	Mburucuyá
Coleoptera		
unidentified	(adults) pollen consumption	Mburucuyá
Chrysomelidae	(larvae) oval holes in buds	Mburucuyá
Thysanoptera	unknown activity inside flowers	Mburucuyá
Heteroptera		
(unidentified)	(nymphs) buds sucking	Mburucuyá
Berytidae Metacanthinae stilt bugs	(adults) legitimately or from below the flowers nectar sucking pollen on mouth parts 1-3 individuals simultaneously in a single flower much time on each flower slow and infrequent movements between flowers	Bs. As. (greenhouse)

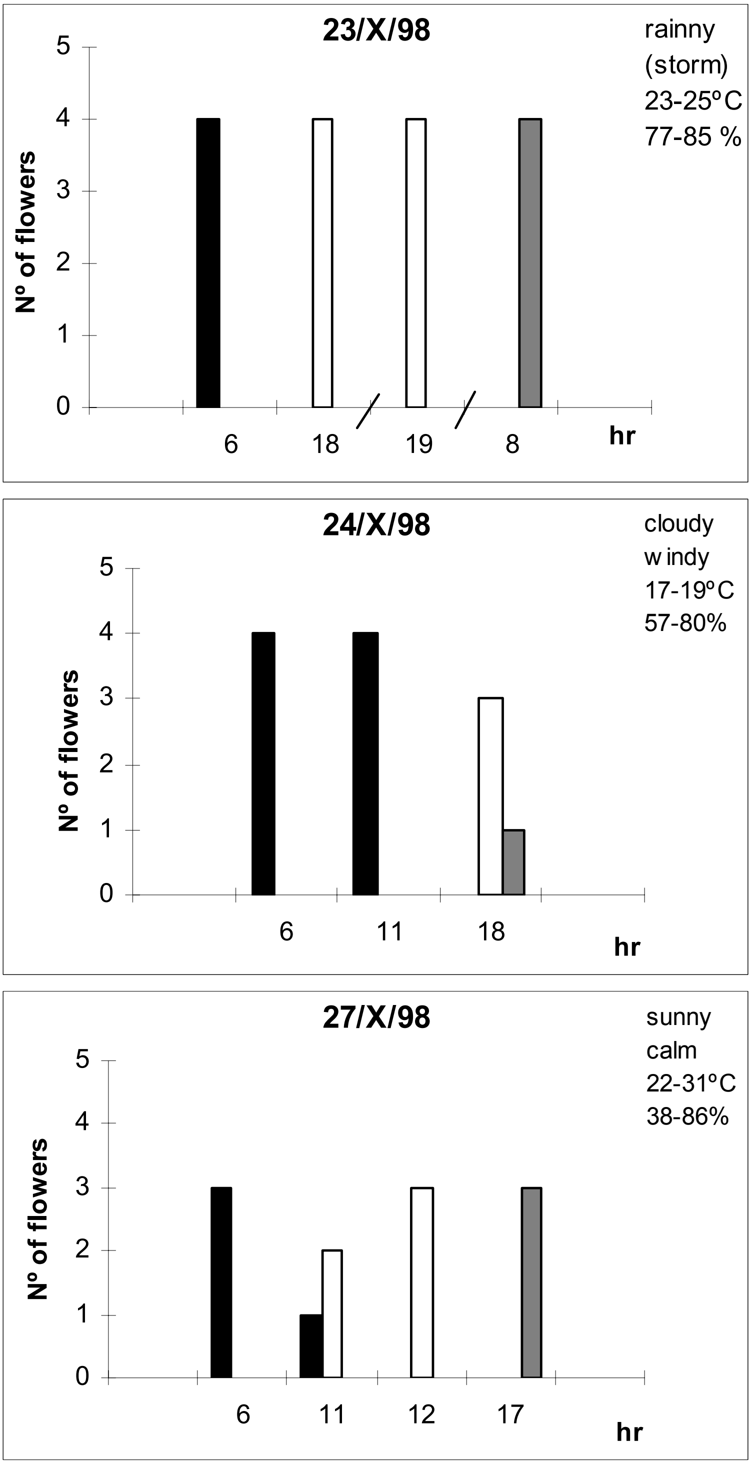


Fig. 2. Floral stages sequence and meteorological conditions. Black: stage 1; white: stage 2; grey: stage 3; lines over x axis separate consecutive days.

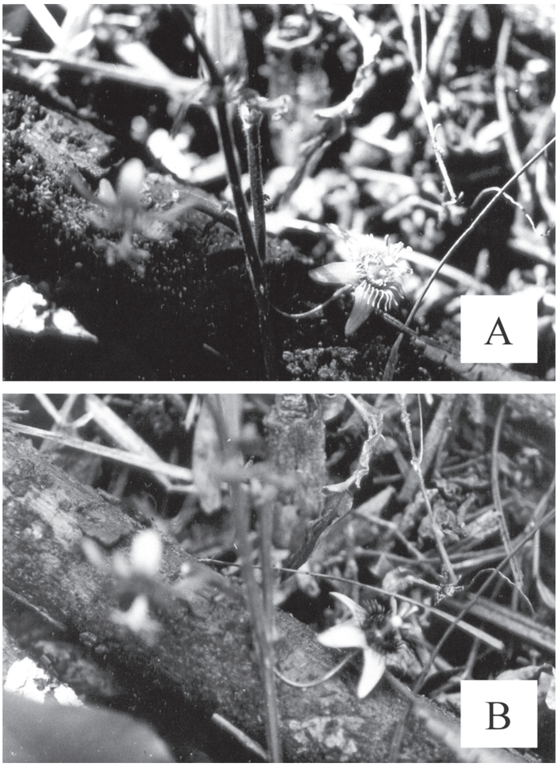


Fig. 3. Reflexion pattern in the UV and visible spectra. **A:** photograph taken in the visible spectrum. **B:** photograph taken in the UV spectrum.

Discussion and Conclusions

Breeding system

The only difference found between localities in this highly variable species (Killip, 1938), was the amount of potential seeds and the fruit shape in the populations from Misiones compared to the one from Corrientes. According to the ISI, this species is self-compatible. The IAS indicates that the autogamy is partial, but, as more than 30 % of fruits were obtained by induced self-pollination, *P. suberosa* would belong to class 2 (highly self-compatible) mentioned by Dafni (1992). The RE was ca. 1; although it is nearly the double of the mean obtained by Ruiz Zapata & Kalin Arroyo (1978) for three self-compatible species, it falls in the ranges given by these authors, compared to the RE of self-incompatible species. The major RRS was obtained when self-pollination was induced, increasing up to nearly the double, which suggests that insufficient pollen is deposited by spontaneous self-pollination (which is evident as only the stigmas edges are touched by the anthers during the usual movements of the reproductive cycles) and also by pollinating agents. So there was quantitative pollen and pollinators' limitation. The reproductive output would be enhanced if pollinators collaborate, at least, with geitonogamous pollination. The lower RRS produced by induced xenogamy respect to self-pollination suggests qualitative pollen limitation among the plants used for this experiment, in accordance to the partial autogamous compatibility detected. The percentages of fruits obtained by Koschnitzke & Sazima (1997) from natural pollination, induced self- pollination and induced xenogamy were lower, similar and higher than the ones from this work, respectively. This last value suggests that they used compatible genotypes for the crossings. Nevertheless, it is difficult to explain the low production of fruits by natural pollination in spite of the pollinators they observed.

The ISI, as well as the IAS and RE, showed each other the same indications either they were calculated using fruits or seeds (either at the greenhouse or in the field for the case of RE), which confirms the absence of significant differences between the fruitful treatments both in fruits and seed production.

Passiflora suberosa flowers present some of the characteristics from outcrossing species and some from selfing ones listed by Ornduff (1969) and Wyatt (1983). Outcrossers features are: not all the ovules fertilized, herkogamy, exposed pollen, extrorse anthers, exerted styles, well defined stigma, nectar and nectar guides present, whereas selfers are: small, odourless, little nectar, greenish, as well as reduced floral pieces (petals absent) and peduncles shorter than the ones of some outcrosser relatives. Compared against other *Passiflora* species, *P. suberosa* self-compatibility is accompanied by other features of autogamic species: fewer ovules/flower, fewer flowers/plant/day, smaller flowers, smaller plants and, consequently, more hidden by surrounding plants from other species, all these resulting in a less conspicuous attraction that conditions a lower frequency of visits.

Floral features

Flower production. Although all passion flowers seem to have a long flowering period and a relative scarce daily flower production («steady state»), if different species are compared, some differences arise: in *P. suberosa* few flowers open per plant per day, as well as in *P. palmatisecta, P. foetida, P. chrysophylla, P. capsularis, P. misera* (Amela García,

1999) and *P. lutea* (Neff & Rozen, 1995), in contrast to *P. mooreana, P. caerulea, P. urnaefolia* (Amela García, 1999), with gregarious flowering, as Faegri & van der Pijl (1979) assumed for the genus. This difference is obviously related to the plants dimensions, as the specimens from the first group are smaller. The frequency of visits recorded by Amela García (1999) was higher in the species from the second group, probably conditioned, at least in part, by the greater attraction and resources availability.

Floral stages. Stage 1 is staminated, although not always the anthers are opened in this stage. Stage 2 is mainly pistilated (pollen receptor); pollen offered in this stage depends on the removal performed by visitors during the previous stage. Stage 3 is staminated (pollen donor) if there is some pollen left. Owing to the stylar movements, these stages are the product of herkogamy.

Visual pattern. In spite of their apparent little attraction to the human eye, the flowers seem to be more attractive in the UV spectrum. In the visible spectrum, the violet *operculum* and surrounding parts (which must be opened to prevent access to the nectar chamber) contrasts with the yellow green remaining floral parts. A similar and more conspicuous pattern is observed in the UV spectrum, so a circular nectar guide, in accordance to the arrangement of the nectar in the circular channeled chamber, is shown.

Floral dimensions. Although *P. suberosa* was said to be extremely variable regarding these characters (Killip, 1938), only the sepals length and the floral diameter had been measured by Deginani (2001) and Koschnitzke & Sazima (1997), respectively. No other data about floral dimensions related with the potential pollinators or dimensions of the visitors received (Koschnitzke & Sazima, 1997) had been published. The plants observed by Koschnitzke & Sazima (1997) and the ones in the present study had small flowers. As the tube is shallow (pateliform), any visitor that could separate the *operculum* would have access to the nectar, without the necessity of long mouth parts.

Visitors, pollinators and reproduction

Regarding their activity, few of the scarce visitors observed on the flowers seem to pollinate this species and, if they do so, due to their low frequency, the pollination would be infrequent. The contact of the Formicidae with the reproductive cycles in phase 2 may be rare as the nectar retention between the *operculum* and *radii* is unusual, so most of the times they must steal nectar. The Andrenidae bees and the adult beetles steal pollen. The unidentified bugs sucked sap. The stilt bugs may be nectar and pollen thieves, as the probability of pollen transfer is very low considering their behavior; at least they must transfer mainly geitonogamous pollen; the majority of the species of Berytidae are thought to be phytophagous, but at least some representatives of certain genera are in part predatory (Schuh & Slater, 1995).

Floral visitors' activity was concentrated in the grasslands of more conspicuous flowering species, fully and constantly exposed to sun and to light for longer periods (especially hymenopterans), or in the sunny forest patches (lepidopterans), both at Mburucuyá National Park and at P. Gentilini Agrothecnic School; the few active insects in the surrounding plants in the forest (*Apis mellifera*, Vespidae, *Bombus* sp., Tabanidae and other Diptera) were obviously not interested in *P. suberosa* flowers. Similarly, the hymenopterans, perhaps capable of pollinating this species, that entered into the greenhouse, were not seen on *P. suberosa* flowers. Taking in account that no fruits were obtained by natural allogamy in the greenhouse, it could be suspected that all the reproductive output is due to self-pollination. Nevertheless, the RE at both sites indicates that the half of the reproductive output is produced by natural pollination (which in compatible species includes xenogamy, geitonogamy and automatic selfing). The fruits obtained by natural pollination might thus had been produced by these 3 ways, although the natural xenogamous and geitonogamous components of this output are not been detected. The scarcity of pollinators seems not to be due to having studied this species at the south extreme of its distribution range. Koschnitzke & Sazima (1997) reported low frequency of pollinators' visits (wasps and small bees) of this species, even the study was carried out in a Brazilian preserve. Other observations on *P. suberosa* flower visitors restrict to the Galápagos isles. McMullen (1985) observed moderate activity of *X. darwini* on *P. suberosa* var. *galapagensis* in Santa Cruz, but it was not clarified if that activity resulted in pollination either. In Pinta, where there are no populations of *Xylocopa darwini* (the unique himenopteran in the archipelago), *P. suberosa* is a nectar source for the finch *Geospiza fuliginosa* during the dry season (Schluter, 1986). Despite the flowers were reported by this author as large (although no measurements were given), the coloration is equal to the one here described (so not the one that commonly attracts birds), and the visits are probably due, besides the absence of *X. darwini* (that seems to pollinate), to the scarcity of other source. The cited author did not mention what effect the finch visits had to the flowers. Other factor conditioning the low frequency of visits could be the partially shaded environments occupied by this species, as solar irradiance influences the foraging responses in some insects (Herrera, 1995).

Anthesis and meteorological conditions

Koschnitzke & Sazima (1997) also observed that the anthers were dehiscent after the flowers had opened (1 hour later), in contrast to most other *Passiflora* species (Amela García, 1999). The late and asynchronous pollen exposition in some flowers (probably due to slow desiccation of the endotecium) might have occurred because of the high humidity and long exposition to the shade in the forests where this species was studied. This delayed dehiscence may be frequent in the wet environments where *P. suberosa* grows. In these flowers, with a neutral first phase without any reproductive cycle available for pollinators, it would not occur dichogamy, as stigmas and anthers are exposed at the same time in stage 2. In this case, the probability that pollinators transfer autogamic pollen is greater. The longer anthesis during cloudy and fresh days, and even longer on rainy days, might constitute a strategy that increases the probability of visits to the flowers during daylight meanwhile there were flowers in stage 2 or flowers in stages 2 and 3 in the population, as the frequency of visits decreases with those meteorological conditions. During this delayed anthesis, the possibility of nocturnal pollination might be discarded, as the flowers do not exhibit attractive traits for nocturnal visitors.

Floral syndrome and breeding strategy

Having in account the perpendicular surface to the floral axis that the sepals and *radii* form (where insects can land), the opaque coloration, the nectar guides in the visible and UV spectra, the hidden nectar at a shallow depth, the relatively easy access to the nectar (as the *operculum* is plicate), the high nectar sugars concentration and the small floral dimensions, the flowers are plausible to be pollinated by small bees and wasps, pollinators observed by Koschnitzke & Sazima (1997).

Some isolated characteristics of flowers pollinated by wasps have been mentioned (Faegri & van der Pijl, 1979) but a wasp syndrome has not been defined, as it seems not to occur. The partially dependence on nectar of the adult predators (even when they have finished the brood-rearing season), the lack of dependence on nectar or pollen of the brood and of a seasonal preference for the flowers (after the nursing period) (Faegri & van der Pijl, 1979) may have contributed not to develop a wasp syndrome. Ramírez (2004) noticed that wasps always appeared as complementary agents in 28 of 155 pollination systems analyzed. Although many floral characteristics of *P. suberosa* may be related to wasps, and even the flowers are outcrossed by wasps and self-pollinated by bees (Koschnitzke & Sazima, 1997), it would be rather uncertain for the plant to rely only on them. The high degree of autogamy would fill this lack.

The percentage of fruits with seeds obtained by spontaneous autogamy was rather high, if compared with other self-compatible *Passiflora* species (~57 % vs. 20 % in *P. foetida* and *P. capsularis*) (Amela García, 1999). Besides, the flowering throughout the year (Koschnitzke & Sazima, 1997; Deginani, 2001) might constitute a strategy that compensates the scarce attraction (both of the single flower and the plant/s blooming) and, consequently, the low frequency of visits.

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