Floral biology of a population of *Mirabilis jalapa* L. (Nyctaginaceae) from Southern Brazil

Ausileide Alves Leal, Yoko Terada[+] and Maria de Fátima Pires da Silva Machado*

*Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. +In memoriam. *Author for correspondence.*

ABSTRACT. The reproductive pattern of a cultivated population of *Mirabilis jalapa* in the Southern region of Brazil was investigated. Flowers of *M. jalapa* opened for one night, in the early evening at 17h00 and closed early the next morning from 4h30 to 6h00. Anther dehiscence occurred from about 19h30 to 20h30. The presence of pollen grains on the stigma surface was recorded in flowers in which the anther lay at the same level as the stigma or higher. Absence of pollen grains was observed in flowers in which the anther was below the stigma and in previously emasculated flowers. One moth was reported to be the only visitor and possible pollinator of *M. jalapa* flowers throughout the experimental period. Self-pollination was the predominant mode of reproduction in the *M. jalapa* population cultured in the garden of the State University of Maringá.

Key words: self-pollination, four o'clock plant, *Mirabilis jalapa*, anthesis.

RESUMO. Biologia floral de uma população de *Mirabilis jalapa* L. (Nyctaginaceae) do Sul do Brasil. Neste estudo foi investigado o padrão reprodutivo de uma população de *Mirabilis jalapa* cultivada na Região Sul do território brasileiro. A abertura das flores de *M. jalapa* ocorreu no início da tarde, às 17 h, e o fechamento foi na manhã seguinte a partir das 4h30min até as 6 h. A abertura das anteras ocorreu a partir das 19h30min até as 20h30min. Foi observada a presença de grãos de pólen na superfície do estigma das flores cujas anteras estavam no mesmo nível, ou num nível superior ao do estigma. A ausência de grãos de pólen no estigma foi verificada nas flores cujas anteras estavam num nível abaixo do da posição do estigma, bem como no estigma de flores previamente emasculadas. Somente uma mariposa foi observada, durante todo o período dos experimentos, visitando e provavelmente polinizando flores de *M. jalapa*. A autopolinização foi o modo predominante de reprodução na população de *M. jalapa* cultivada no jardim da Universidade Estadual de Maringá.

Palavras-chave: auto-polinização, planta das quatro horas, *Mirabilis jalapa*, ântese.

Mirabilis jalapa (Nyctaginaceae), also known as the four o'clock plant, is a tropical American herb commonly cultivated in North America. It is perennial in the south and warm west and annual in the north. Long before the Spanish Conquest of Mexico it was cultivated by the Aztecs for its medicinal properties and for its showy fragrant flowers (Le Duc, 1986). *M. jalapa* was a study model for the genetic laws of Mendel and has been explored as material for different studies (Lorenzi, 1991).

The medicinal properties of *M. jalapa* have been well characterized in several studies. A protein purified from the root tubers of *M. jalapa* was confirmed to be an antiviral protein (Kataoka *et al*., 1991, 1992; Wong *et al*., 1992). While the protein has also an inhibitory effect on cell-free protein synthesis and an antiproliferative effect on tumor cells, it inhibits too the mechanical transmission of plant viruses and the in vitro protein synthesis of prokaryotes and eukaryotes (Miyano *et al*., 1992). Antimicrobial peptides from seeds of this plant species have also been detected and characterized (Cammue *et al*., 1992; De Bolle *et al*., 1995).

The floral and reproductive biology of natural and cultivated populations of *M. jalapa* has been mainly studied in material from the North American continent. Self-pollination was described as its primary means of reproduction (Cruden, 1973; Niesenbaum and Schueller, 1997) and in some natural populations hawk-moth pollinators have been reported (Martinez del Rio and Búrquez, 1986).

Since *M. jalapa* plants provide various compounds of medicinal interest, in the present study we investigated the reproductive pattern of a cultivated population of this plant in the Southern region of Brazil. In the traditional popular medicine of the South American continent, particularly in Brazil, the roots, shoots, leaves, fruits, and seeds of *M. jalapa* are employed for different affections (Lorenzi, 1991; Lorenzi and Souza, 1995). Thus, information about the reproductive pattern of this culture is important for implementing experimental procedures. Estimates of plant mating systems are necessary if we are to understand the forces that affect the genetic organization of natural or cultivated plant populations.

Material and methods

The observations reported in the present study were performed on a population of *M. jalapa* bred in Herb Garden of the State University of Maringá, Maringá, state of Paraná, Brazil (554.9 altitude.; 23°25' S, 51°25' W). The study was conducted from November 1998 to March 1999, when the mean temperature recorded at dusk was 34.4 – 20.2°C. All plants were systematically watered twice a day.

Despite the occurrence of a variegate pattern for *M. jalapa* flowers, only plants with fully yellow, red, white or pink flowers were used as samples in the present study. Twenty flowers of each color were used to observe their daily opening and 30 flowers of each color were used to observe the daily opening of their anthers. Each *M. jalapa* flower had five stamens and a single-ovulate ovary.

Randomly selected pollen grains from at least three anthers per flower were tested for viability by propionic carmine (Radford *et al.*, 1974). Stigma receptivity was measured using hydrogen peroxide (20 v) applied to the stigma surface.

The height of stigma (length of style) and anther (length of filament) from 15 pink flowers was measured to observe the dynamic of self-pollination. Occurrence of pollinators was determined by 32 red and 35 pink open flowers which were emasculated to prevent premature self-pollination. The choice of pink and red flowers for these experiments was made at random, for no special reason.

Fruit production was analyzed for flowers submitted to open-pollination, emasculated flowers, and flowers covered with fine mesh bags to assure self pollination.

Results

The flowers of *M. jalapa* opened for one night in the early evening, at 17h00, and closed early the next morning from 4h30 to 6h00. Anther dehiscence occurred from about 19h30 to 20h30 (Figure 1).

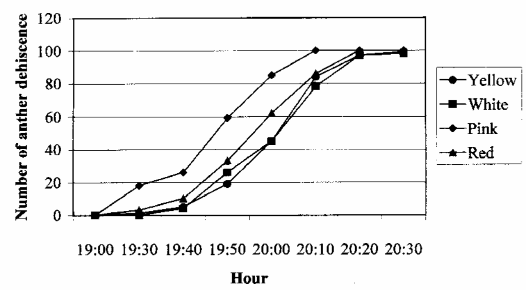

Figure 1. Daily opening of the *Mirabilis jalapa* anthers

The height of stigma and anther was measured in 15 pink flowers at 19h00 and 23h00 (Table 1). Figure 2 shows the anther position below the stigma at 19h00, while at 23h00 anther position was at the same level of the stigma or above it (Figure 3). At 20h00 and 23h00, 52% and 68% of the flowers, respectively, showed the presence of pollen on the stigma surface. In all the flowers anther position was at the same level as the stigma or above it. Absence of pollen grains was observed in 83.4% of the flowers, with anther positions below the stigma. Pollination pattern was also observed for flowers previously covered with fine mesh bags. At 23h00 the presence of pollen grains on the stigma surface was observed in 80% of the flowers in which anther position was at the same level as the stigma.

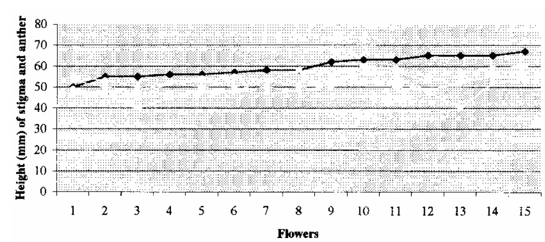

Figure 2. Length (mm) of stigma (white) and anther (black) of *Mirabilis jalapa* flowers measured at 19h00

Analysis of 67 previously emasculated flowers collected prior to anther opening (16h30) and on the next morning (8h00) showed absence of pollen grains on the stigma surface. Pollen grains were contrastingly observed on the stigma surface of 92% of the control flowers used in this experiment. Tests for pollen viability revealed that 88% of the examined pollen grains (n = 2309) showed color and were thus deemed viable (Figure 4). Stigma receptivity monitored hourly from 17h00 to 4h30 of the next morning indicated positive receptivity in all cases.

Table 1. Height of the stigma and anther (mm) of *Mirabilis jalapa* pink flowers

	Height of Stigma and Anther (mm)														
	at 19h00														
Flower	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Stigma	50	55	55	56	56	57	58	58	62	63	63	65	65	65	67
Anther 1	47	46	40	45	48	48	47	48	50	53	50	48	40	52	53
Anther 2	48	46	45	47	48	48	50	48	51	57	52	48	52	55	54
Anther 3	49	46	45	52	50	48	53	50	51	57	55	48	53	55	55
Anther 4	53	50	48	52	53	50	53	50	53	57	57	53	55	56	55
Anther 5	55	53	50	52	54	55	53	58	55	60	59	55	57	59	60

	Height of Stigma and Anther (mm)														
	at 23h00														
Flower	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Stigma	70	62	68	69	70	65	63	61	62	65	67	65	67	62	65
Anther 1	63	55	60	60	65	63	59	60	57	62	59	56	62	56	57
Anther 2	64	55	60	62	67	64	60	63	57	62	61	58	63	57	60
Anther 3	65	60	65	65	70	66	62	65	59	67	61	61	65	59	60
Anther 4	67	60	65	65	70	67	63	65	62	67	62	63	67	60	61
Anther 5	67	60	66	67	72	68	60	65	61	70	63	68	70	61	63

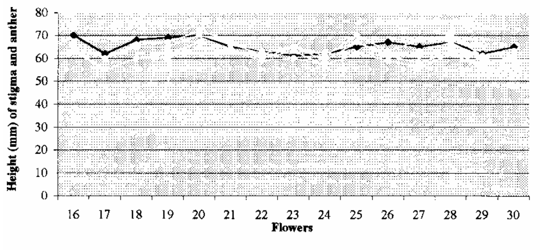

Figure 3. Height (mm) of stigma (white) and anther (black) of *Mirabilis jalapa* flowers measured at 23h00

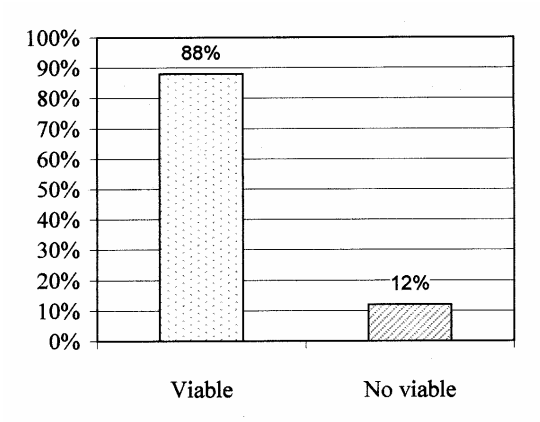

Figure 4. Proportion of viable pollen of *Mirabilis jalapa* flowers

Fruit development was low in open-pollinated flowers, flowers covered with fine mesh bags and emasculated flowers, and tended to decline sharply in emasculated flowers, of which only 5% produced fruits (Figure 5).

From 16h30 to 19h00 the flowers were visited daily by different insects of the genera *Xylocopa*, *Thrips*, *Halictidae* and *Diabrotica*. Since they pierced the base of the petaloid calyx or probed the flowers, always before anther dehiscence, they acted as nectar thieves. Only one moth was recorded to be a visitor of *M. jalapa* flowers throughout the experimental period (from November 1998 to March 1999). The moth showed flying activity after 19h30 and introduced its proboscis into one yellow flower at 20h30. Pollen grains were present in this moth; however, it was not reported having introduced its proboscis into other flowers.

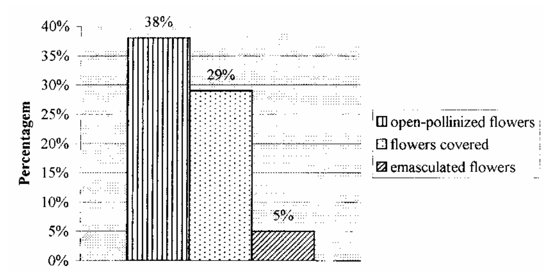

Figure 5. Proportion of *Mirabilis jalapa* fruit obtained from open-pollinated flowers, flowers covered with mesh bags, and emasculated flowers

Discussion

Two species of hawk-moths (*Erinnyis ello* and *Hyles lineata*) have been reported as the main pollinators of *M. jalapa* (Martínez del Rio and Búrquez, 1986); however, self-pollination is the predominant mode of reproduction in the *M. jalapa* population cultured in the Herb Garden of the State University of Maringá. The presence of pollen on the stigma surface in flowers showing the anther's position at same level as the stigma or above it, as well as in the flowers previously covered, is evidence of the self-pollination process. Although flower behavior (opening flowers and anthers) and flower visitors (acting as nectar thieves) are in accordance with data reported for other *M. jalapa* populations (Martínez del Rio and Búrquez, 1986), insect pollinators seem to be rare in the population analyzed in the present study.

The influence of temperature and nectar production on the pollination of *M. jalapa* has been documented for a Central Mexico population, where the breeding structure of these populations seemed to depend on temperature conditions. They were mainly autogamous on cold night and allogamous on warm ones. The hawk-moths that pollinate the plants failed to fly at dusk, at approximately below 13°C. Since the mean dusk temperatures registered in the present study were 30.4-20.2°C, a cold temperature might not be the factor responsible for the absence of moth pollinators. In some natural populations where the hawk-moth pollinator is nearly extinct, self-pollination has also been described as the sole means of reproduction (Niesenbaum and Schueller, 1997).

According to McCracken and Selander (1980), selfing could be a protective mechanism for genetic material accumulated along the evolutionary history of the species. In such instances, this reproductive pattern seems to be an effective mechanism for the maintenance of an environmentally adapted genotype, and therefore, a mechanism for evolution preservation. However, the predominant self-pollination may be one of the factors responsible for the low rate of fruit development observed in *M. jalapa* plants grown in the Herb Garden of the State University of Maringá. Self-pollination is a reproductive mechanism that leads to a loss of genetic variability and affects the adaptive capacity of a species by altering the number of homozygous loci. There is some evidence that selection operates against inbred progeny in self-compatible species (Hagman and Mikkola, 1963; Sorenson and Milles, 1974; Lindgren, 1975). The selfed seeds presumably have lower rates of survival than outcrossed seeds (Sorenson, 1982). Schoen (1982) demonstrated that populations of *Gallia achilleifolia* with low rates of outcrossing are genetically different from those with higher outcrossing rates. Self-compatible species generally result in inbreeding depression (Schemske, 1983). Based on their theoretical analyses, Schemske and Lande (1987) argued that species with mixed mating systems are relatively rare. *M. jalapa* might be one of these rare examples of mixed mating system.

Pollen, nectar, perfume, color of the flowers are a few of the requirements of angiosperm groups used to attract pollinators (Endress, 1996). *M. jalapa* has flower characteristics such as crepuscular anthesis, strong sweet odors, and tubular flowers for the phalaenophily syndrome described by Faegri and Pill (1976). However, these devices in *M. jalapa* flowers to attract pollinators are a contrast to the absence of pollinator agents for the populations cultivated. Such result cannot be easily explained since there are various mechanisms to avoid exclusive extremes in plant biology reproduction, where there is a compromise of quantity vs. quality in the production of gametophytes, seeds and seedlings (Wilson, 1983), resulting in a flexible balance between extreme conditions. Since the study time was short (during a single season) and *M. jalapa* plants were grown in only one place, similar studies should be extended to populations under different circumstances as well as to populations from different Brazilian regions for a better understanding of the biology and diversity of the *M. jalapa* plants grown on the tropical and subtropical continent.

In the case of *M. jalapa* plants analyzed in the present study, depending on the experimental goal, artificial pollination should be employed to increase outcrossing rates and genetic variability in this population. On the other hand, the genetically uniform plants of *M. jalapa* are a suitable source for industrial procedures of extraction of their medicinal compounds. Extraction protocols may be easily standardized using genetically uniform materials. In order to obtain a larger number of *M. jalapa* plants cultured in the present study, vegetative propagation or tissue culture techniques, for example, may be employed for rapid multiplication of the species.

References

CAMMUE, B. P. *et al.* Isolation and characterization of a novel class of plant antimicrobial peptides from *Mirabilis jalapa* L. seeds. *J. Biol. Chem.,* Bethesda, v. 267, p. 2228-2233, 1992.

CRUDEN, R. W. Reproductive biology of weedy and cultivated *Mirabilis* (Nyctaginaceae). *Am. J. Bot.,* Columbus, v. 60, p. 802-809, 1973.

DE BOLLE, M. F. *et al.* Cloning and characterization of two cDNA clones encoding seed-specific antimicrobial peptides from *Mirabilis jalapa* L. *Plant Mol. Biol.,* Dordrecht, v. 28, p. 213-721, 1995.

ENDRESS, P. K. *Diversity and evolutionary biology of tropical flowers*. Cambridge: University Press, 1996.

FAEGRI, K.; PILL, L. *The Principles of pollination ecology*. New York: Pergamon Press, 1976.

HAGMAN, M.; MIKKOLA, L. Observations on cross-, self-, and inter specific pollination in *Pinus peuce* Griseb. *Silvae. Genet.,* Frankfort on the Main, v. 12, p. 73-79, 1963.

KATAOKA, J. *et al.* DNA sequence of Mirabilis antiviral protein (MAP), a ribosome-inactivating protein with an antiviral property, from *Mirabilis jalapa* L. and its expression in *Escherichia coli*. *J. Biol. Chem.,* v. 266, p.8426-8430, 1991.

KATAOKA, J. *et al.* Adenine depurination and inactivation of plant ribosomes by an antiviral protein of *Mirabilis jalapa* (MAP). *Plant Mol. Biol.,* Dordrecht, v. *20*, p. 1111-1119, 1992.

LE DUC, A. A revision of *Mirabilis* section *Mirabilis* (Nyctaginaceae). *Sida Contrib. Bot.,* Fort Worth, v. 16, p. 613-648, 1986.

LINDGREN, D. The relationship between self-fertilized, empty seeds and seeds originating from selfing as a consequence of polyembryony. *Stud. For. Suec.*, Uppsala, p. 126, 1975.

LORENZI, H. *Plantas Daninhas do Brasil*. 2. ed. São Paulo: Nacional, 1991.

LORENZI, H.; SOUZA, H. M. *Plantas ornamentais no Brasil*. Nova Odessa: Plantarum, 1995. p. 720.

MARTINEZ del RIO, C.; BURQUEZ, A. Nectar production and temperature dependent pollination in *Mirabilis jalapa* L. *Biotropica*, Lawrence, v. 18, p. 28-31, 1986.

McCRACKEN, G. F.; SELANDER, R. K. Self-fertilization and monogenic strains in natural populations of terrestrial slugs. *Proc. Natl. Acad. Sci. USA.,* Washington, DC., v. 77, p. 684-688, 1980.

MIYANO, M. *et al.* Crystallization and preliminary X-ray crystallographic analysis of *Mirabilis* antiviral protein. *J. Mol. Biol*., London, v. 226, p. 281-283, 1992.

NIESENBAUM, R. A.; SCHUELLER, S. K. Effect of pollen competitive environment on pollen performance in *Mirabilis jalapa* (Nyctaginaceae). *Sex Plant Reprod.,* Berlin, v. 10, p. 101-106, 1997.

RADFORD, A. E. *et al. Vascular Plants Systematics*. New York: Harper & Row, 1974.

SCHEMSKE, D. W. Breeding system and habitat effects on fitness components in three Neotropical *Costus* (Zingiberaceae). *Evolution,* Lawrence, v. 37, p. 523-539, 1983.

SCHEMSKE, D. W.; LANDE, R. On the evolution of plant mating systems. *Am. Nat.*, Chicago, v. 130, p. 804-806, 1987.

SCHOEN, D. J. The breeding system of *Gallia achilleifolia*: variation in floral characteristics and outcrossing rate. *Evolution,* Lawrence, v. 36, p. 352-360, 1982.

SORENSON, F. C. The roles of polyembryology and embryo viability in the genetic system of conifers. *Evolution,* Lawrence, v. 36, p. 725-733, 1982.

SORENSON, F. C.; MILES, R. S. Self-pollination effects on Douglas fir and Ponderosa pine seeds and seedlings. *Silvae. Genet*. Frankfort on the Main, v. 23, p. 135-138, 1974.

WILSON, M. F. *Plant reproductive ecology*. New York: Wiley, 1983.

WONG, R. N. *et al.* Characterization of Mirabilis antiviral protein – a ribosome inactivating protein from *Mirabilis jalapa* L. *Biochem. Int.*, Marrickville, v. 28, p. 585-593, 1992.

Received on December 19, 2000.

Accepted on February 15, 2001.