

Permanent stigma closure in Bignoniaceae: Mechanism and implications for fruit set in self-incompatible species

Paulo Milet-Pinheiro^{a,*}, Airton Torres Carvalho^a, Peter G. Kevan^b, Clemens Schlindwein^c

^a*Programa de Pós-Graduação em Biologia Vegetal, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, 50670-901 Recife, PE, Brazil*

^b*Department of Environmental Biology, University of Guelph, Guelph, Ont., Canada N1G 2W1*

^c*Departamento de Botânica, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, 50670-901 Recife, PE, Brazil*

Received 23 October 2007; accepted 30 November 2007

Abstract

The Bignoniaceae possess sensitive bilobate stigmas that close after stimulation. We determined factors involved in stigma closure and reopening in four species of Bignoniaceae from the National Parks of Catimbau, Northeastern Brazil (*Anemopaegma laeve*, *Arrabidaea limae*, *Jacaranda rugosa* and *Tabebuia impetiginosa*). The study showed that any mechanical touch promoted the stigmatic closure. Only the deposition of viable con-specific pollen (self- and cross-pollen), however, maintained stigmas permanently closed. In *Arrabidaea limae* part of the stigmas reopened after self- and cross-pollination. After mechanical stimulation, deposition of pollen from other plant species, of dead con-specific pollen or of sand, the stigma lobes opened again after 38 to 68 min. The definitive closure may have a negative effect on the reproductive success of the involved plants, because the stigmas are permanently unavailable for pollen deposition. In self-incompatible species with mass-flowering blooming pattern, where pollinators promote high levels of geitonogamy, definitive stigma closure causes low fruit set.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: Sensitive stigmas; Pollination; Self-incompatibility; Geitonogamy; Mass flowering; Northeastern Brazil

Introduction

Sensitive stigmas occur exclusively in the order Scrophulariales (traditional circumscription), especially in the families Bignoniaceae, Lentibulariaceae, Martyniaceae and Scrophulariaceae (Newcombe, 1922, 1924). Common characteristics of plant species with sensitive stigmas are hermaphrodite flowers, tubular corollas and bilobate stigmas (Fetscher and Kohn, 1999; Newcombe

1922, 1924). Among Bignoniaceae-sensitive stigmas are widespread. Stevens (1994), found this phenomenon in all 18 Bignoniaceae species studied from the Cerrado of Brazil.

The evolutionary and functional significance of sensitive stigmas were studied since the 19th century (Darwin, 1876; Newcombe, 1922, 1924). The function of stigmatic closure was associated with prevention of self-pollination and facilitation of cross-pollination (Fetscher and Kohn, 1999; Gottsberger and Silberbauer-Gottsberger, 2006; Newcombe, 1922, 1924; Ritland and Ritland, 1989; Stevens, 1994), increase in capture and

*Corresponding author. Fax: + 55 81 21268348.

E-mail address: miletpinheiro@hotmail.com (P. Milet-Pinheiro).

retention of pollen grains (Thieret, 1976), reduction of the interference between reception and export of pollen within flowers (Fetscher, 2001; Fetscher et al., 2002; Webb and Lloyd, 1986), increase of pollen export (Fetscher and Kohn, 1999; Fetscher et al., 2002) and finally, providing more suitable conditions for pollen grain germination (Newcombe, 1922, 1924). These proposed functions are not mutually exclusive, and stigma closing may have different consequences depending on the species.

In several species the stigma closure is permanent (Bertin, 1982; Newcombe, 1922, 1924; Singh and Chauhan, 1996; Yang et al., 2004), but it is not known which factors determine permanent closure. We asked the following questions: (1) Which stimuli determine stigma closing and reopening? (2) What determines permanent stigma closure? (3) Does the mechanism cease after several closings and re-openings? (4) Does the closing response follow the same pattern in different species?

Material and methods

Study site

The study was carried out in 2005 in the National Park of Catimbau, a nature reserve which covers 62,000 ha in the municipalities of Buíque, Tupanatinga and Ibimirim, in the state of Pernambuco, northeastern Brazil (Andrade et al., 2004). The vegetation of the Park is composed of evergreen shrubs and small trees among which typical species of the surrounding Caatinga intermingle, the common deciduous vegetation type of semi-arid NE-Brazil (Rodal et al., 1998). Mean annual temperature and precipitation are, respectively, 25 °C and 1095.9 mm and the rainy season is from April to June (Sudene, 1990). In this Nature Reserve, at least five species of Bignoniaceae occur (Andrade et al., 2004; Gomes et al., 2006; Rodal et al., 1998) of which we made observations on *Jacaranda rugosa* Gentry, *Tabebuia impetiginosa* (Mart. ex DC.) Standl., *Anemopaegma laeve* DC. and *Arrabidaea limae* Gentry.

Species studied

Tabebuia impetiginosa, called *Pau d'arco* or *Ipê-roxo*, is a tree with a wide distribution in South America, occurring from NW-Mexico to NW-Argentina. *Jacaranda rugosa* is an endemic shrub of the National Park of Catimbau and its surroundings, showing steady-state flowering and large staminodes typical of the genus (Gentry, 1980, 1992; Morawetz, 1982). *Anemopaegma laeve* occurs from Minas Gerais to Piauí and is a woody vine with light yellow flowers and a steady-state flower-

ing pattern. *Arrabidaea limae* is a woody vine with purple flowers, restricted to NE-Brazil. The flowers of all species are large, hermaphroditic and show a bilobate sensitive stigma (Fig. 1). All species are pollinated by medium to large sized *Euglossa* and *Centris* bees (Carvalho et al., 2006; Milet-Pinheiro and Schlindwein, 2008, unpublished).

Characterization of stigma closure

Two experiments were made to characterize the stigma closure of the four species studied. In the first, we verified to which stimulus type the stigmatic closure respond and what determines permanent closing. The stigmata were treated in six different ways: (1) mechanical touch of the stigma lobes; (2) deposition of con-specific pollen grains from different individuals (cross-pollen); (3) deposition of con-specific pollen from the same individual (self-pollen); (4) deposition of some sand grains; (5) deposition of *Ipomoea* pollen (Convolvulaceae) and (6) deposition of dead con-specific cross-pollen. The pollen grains for this last treatment were killed by heating them in a container in a water bath at 65 °C for one hour. Pollen grain deposition and mechanical touches were made using fine-tipped entomological forceps at the beginning of the day of study. All flowers used in the experiment had been previously bagged while in bud to exclude visits from anthophilous animals. Each of these six treatments were made at the same plant and repeated in six (*T. impetiginosa*, *J. rugosa*) or 15 (*Anemopaegma laeve* and *Arrabidaea limae*) individuals. The position of the stigma was recorded soon after stimulation, two hours after stimulation, at the end of the day and on subsequent days in species with prolonged flowering. Fruit set of these treatments was recorded. Moreover, we determined natural fruit set by marking open flowers of *Arrabidaea limae* ($n = 1500$ flowers), *Anemopaegma laeve* ($n = 72$), *J. rugosa* ($n = 260$) and *T. impetiginosa* ($n = 180$). The breeding systems of these species are treated elsewhere (Carvalho et al., 2006; Milet-Pinheiro and Schlindwein, 2008, unpublished). Our studies showed that *Anemopaegma laeve*, *J. rugosa* and *T. impetiginosa* are obligate outcrossers while *Arrabidaea limae* is a facultative xenogamic species.

In the second experiment, we determined the time needed for stigma closure and reopening after a mechanical touch. For that, 15 flowers of five individuals of each species of plants were previously bagged to avoid insect visits. The stigma lobes were mechanically stimulated and, after reopening, additional mechanical touches were made to verify the continuity of the stigmatic sensitivity and velocity of responses. In *T. impetiginosa* mechanical stimulations to stigmas of flowers of the first, second and third day of anthesis were

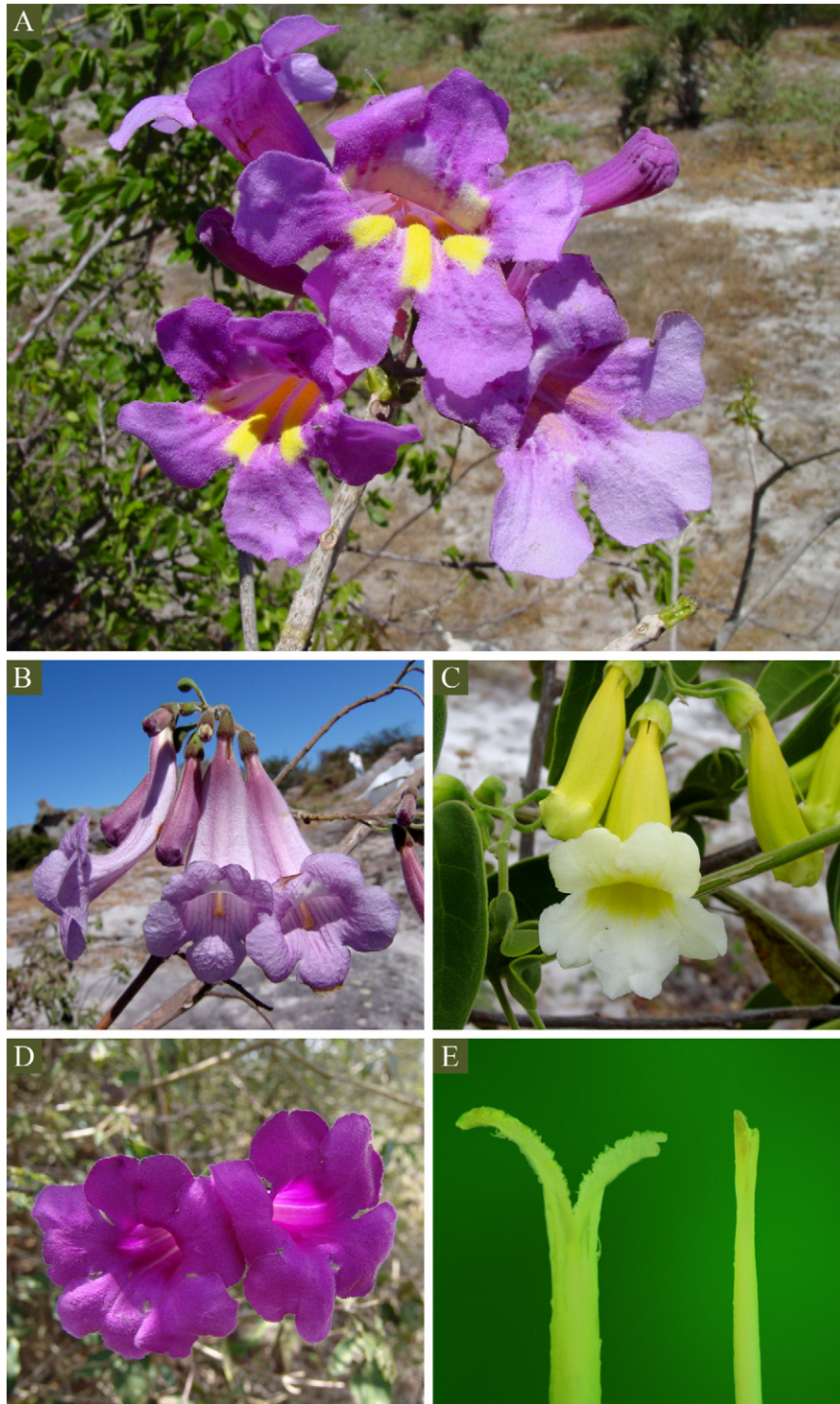


Fig. 1. Flowers of the studied species of Bignoniaceae. (A) *Tabebuia impetiginosa*, (B) *Jacaranda rugosa*, (C) *Arrabidaea limae* and (D) *Anemopaegma laeve* and (E) Bilobate stigma of *Arrabidaea limae* before (left) and after mechanical stimuli (right).

made to check for possible differences in the time needed for closure among flowers of different age. ANOVA was used to test if the differences among the closing times of

flowers of different ages were significant. Tukey's test was used to compare the differences among the pairs, and the Kolmogorov–Smirnov test to assess normality

Table 1. Position of stigma lobes in flowers of four Bignoniaceae species after six kinds of stimulation: mechanical stimulation, deposition of cross-pollen, deposition of self-pollen, deposition of sand, deposition of *Ipomoea* pollen grains (Convolvulaceae) and deposition of unviable cross-pollen

Treatment/species	Position of stigmatic lobes									
	Soon after stimulus		2 h after stimulus		End of the day		1 day after stimulus		2 days after stimulus	
	CS	OS	CS	OS	CS	OS	CS	OS	CS	OS
<i>Tabebuia impetiginosa</i>										
Mechanical touch	6	0	0	6	0	6	0	6	0	6
Hand cross-pollination	6	0	6	0	6	0	6	0	6	0
Hand self-pollination	6	0	6	0	6	0	6	0	6	0
Sand deposition	6	0	0	6	0	6	0	6	0	6
<i>Ipomoea</i> sp. pollen deposition	6	0	0	6	0	6	0	6	0	6
Deposition of unviable cross-pollen	6	0	0	6	0	6	0	6	0	6
<i>Jacaranda rugosa</i>										
Mechanical touch	6	0	0	6	0	6				
Hand cross-pollination	6	0	6	0	6	0				
Hand self-pollination	6	0	6	0	6	0				
Sand deposition	6	0	0	6	0	6				
<i>Ipomoea</i> sp. pollen deposition	6	0	0	6	0	6				
Deposition of unviable co-specific pollen	6	0	0	6	0	6				
<i>Anemopaegma laeve</i>										
Mechanical touch	15	0	1	14	0	15	0	15	0	15
Hand cross-pollination	15	0	15	0	15	0	15	0	15	0
Hand self-pollination	15	0	15	0	14	1	14	1	14	1
Sand deposition	15	0	6	9	3	12	0	15	0	15
<i>Ipomoea</i> sp. pollen deposition	15	0	4	11	0	15	0	15	0	15
Deposition of unviable co-specific pollen	15	0	7	8	1	14	0	15	0	15
<i>Arrabidaea limae</i>										
Mechanical touch	15	0	2	13	2	13	0	15	0	15
Hand cross-pollination	15	0	15	0	11	4	6	9	5	10
Hand self-pollination	15	0	8	7	5	10	2	13	1	14
Sand deposition	15	0	5	10	1	14	0	15	0	15
<i>Ipomoea</i> sp. pollen deposition	15	0	1	14	1	14	0	15	0	15
Deposition of unviable co-specific pollen	15	0	0	15	0	15	0	15	0	15

CS – closed stigma and OS – open stigma. The flowers of *Jacaranda rugosa* open for just 1 day, and those of the other species 3–7 days.

of the samples (Zar, 1999). The tests were made using Statistica version 6.0 (Statsoft, 2001).

Results

Stigma closure resulted from any stimulus we used in flowers of all species studied (Table 1). Definitive closure, however, occurred only after deposition of viable con-specific pollen on the stigma in three of the four studied species. The stigmatic lobes remained closed after deposition of both self- and cross-pollen. In *Arrabidaea limae* most of the stigmas reopened even after the deposition of con-specific self- or outcross-pollen grains (Table 1). Controlled pollination showed that flowers set fruit only after cross-pollination.

The time needed for stigma closure after a stimulus was long in *Anemopaegma laeve* and *Arrabidaea limae*, on average 69 and 76 s, respectively, and short in *T. impetiginosa* (16.4 s) and *J. rugosa* (23 s) (Fig. 2). The stigmas took on average between 38 (*T. impetiginosa*) and 66 min (*J. rugosa*) to reopen after the stimulus (Fig. 3).

Repetitive mechanical stimuli after every stigmatic reopening showed that the closing and reopening mechanism in all the species did not cease and the stigmas always reopened.

Mechanical stimuli applied to the stigma of *T. impetiginosa* on the first, second and third day of anthesis showed that the stigmas remained sensitive throughout anthesis. The time needed for closing, however, increased from 13 to 130 s from first day to third day flowers (Fig. 4).

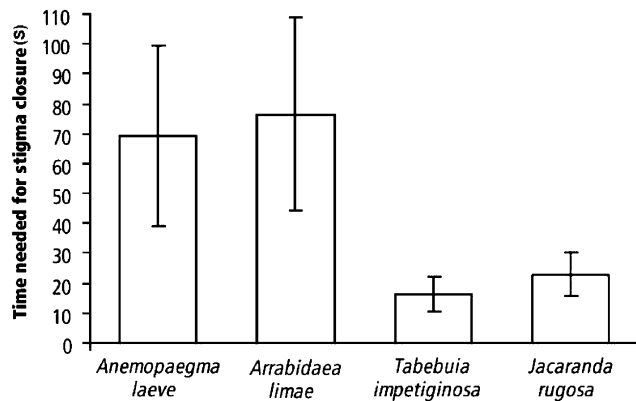


Fig. 2. Time needed for stigma closure after mechanical stimuli in four species of Bignoniaceae, average and standard deviation ($n = 15$).

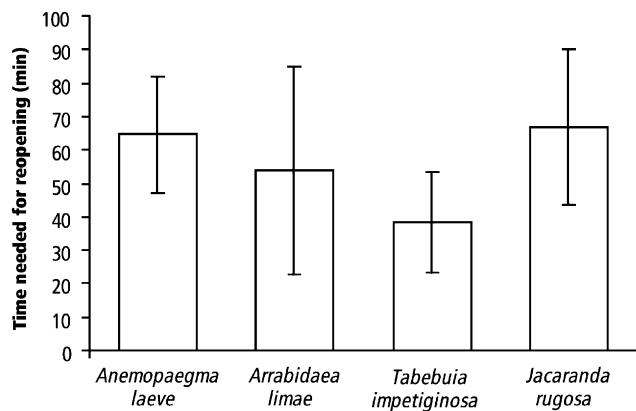


Fig. 3. Intervals of reopening of stigmas after being closed by mechanical stimuli in four species of Bignoniaceae, average and standard deviation ($n = 15$).

Discussion

In three of the four species studied, con-specific pollen grains were recognized on the stigmatic surface and caused definitive stigmatic closure, independently if stigmatic lobes were pollinated by self- or cross-pollen. Like mechanical stimuli alone, deposition of sand, non-conspecific pollen grains and dead con-specific pollen on the stigmatic lobes all resulted in reopening of the stigmatic lobes. Chemical specific marker substances which are recognized at the stigmatic surface may be released only by living con-specific pollen grains. Sporophytic markers in the pollenkit are improbable because only living pollen caused irreversible closing. Although several studies have shown permanent stigmatic closure after cross-pollination (James and Knox, 1993; Richardson, 2004; Singh and Chauhan, 1996; Stephenson and Thomas, 1977), only Yang et al. (2004) have detected permanent closure also after self-

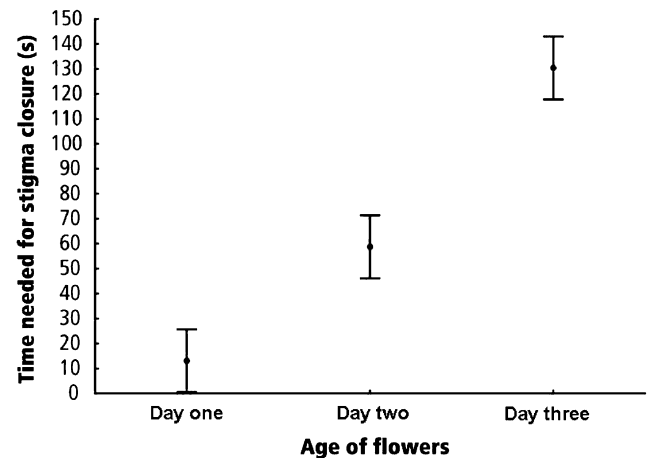


Fig. 4. Duration of interval between mechanical stimulus and stigma closure on first, second and third day flowers in *Tabebuia impetiginosa* ($n = 10$; average and confidence interval). The time needed for stigma closure in flowers of different days was significantly different ($F = 92.49$; $g.l = 2$; $p < 0.00001$). Tukey's test showed significant difference among all treatments ($p < 0.001$).

pollination in *Campsis radicans* (Bignoniaceae) from North America.

Our results indicate that, in Bignoniaceae, stigmatic deposition of self-pollen commonly results in permanent stigma closure. That is surprising because three of the species we studied are self-incompatible, a common feature in the Bignoniaceae (Bawa, 1974; Frankie et al., 1983; Gentry, 1990; Stevens, 1994), and ineffective geitonogamous pollen transfer that causes stigmatic unavailability to effective xenogamy would seem maladaptive. More curious is our finding that in *Arrabidaea limae* only two-thirds of the stigmas remained closed after deposition of cross-pollen but more than 90% after deposition of self-pollen. We have no explanation for that difference. Furthermore, fruit set in *Arrabidaea limae* was near zero (0.01%; $n = 1500$) (Carvalho et al., unpublished). In several Bignoniaceae the time needed for stigmatic closure is longer than the foraging durations of pollinators in the flowers (Bertin, 1982). That applies to two of the species we studied, *J. rugosa* and *T. impetiginosa* for which the durations of flower visits were short, lasting on average 9.8 s (Milet-Pinheiro and Schlindwein, 2008, unpublished), yet stigmatic closure took on average 16.4 s in *T. impetiginosa* and 23 s in *J. rugosa*. Thus, stigma closure can not be ascribed to reducing the likelihood of autogamous self-pollination, as suggested for other species of Bignoniaceae (Fetscher and Kohn, 1999; Newcombe, 1922, 1924; Ritland and Ritland, 1989).

When pollinators make numerous flower visits in sequence at the same plant, the flow of cross-pollen is low and deposition of self-pollen on the stigmata high.

High levels of geitonogamy through those means are common in mass-flowering species (Bawa, 1974; Gentry, 1978) like those of big-bang, multiple-bang and also cornucopian flowering patterns. Thus, in self-incompatible Bignoniaceae, only the first pollinator visits to flowers of individual plants may result in fruit set. Subsequent visits increase the flow of geitonogamous pollen, cause stigmatic closure and, consequently, make stigmas unavailable for later depositions of cross-pollen.

Those characteristics must cause low fruit set. In fact, Stevens (1994) studying mass-flowering species of Bignoniaceae from Cerrado showed a very low fruit production: *Tabebuia rosea-alba* (0.9%), *T. impetiginosa* (1.6%), *T. serratifolia* (0.3%), *T. caraiba* (0.27%), *T. ochracea* (0.39%) and *T. rosea* (0.18%). Mass-flowering plants invest strongly in visual attraction and, in general, produce far more flowers than would be expected to be required for seed-set commensurate with sustainable population levels of flowers. Therefore, low fruit set may be part of the reproductive strategy of these species, especially because they produce relatively large fruits with numerous seeds. In *Hancornia speciosa* Gomes (Apocynaceae), a mass-flowering, self-incompatible species with a highly specialized pollination mechanism, only the first two visits of flowers in a visit sequence in the same plant individual were efficient in forming fruit (Pinto et al., 2008). The following visits promoted only geitonogamous pollen flow and did not result in fruit set.

In continuously flowering tropical Bignoniaceae, on the other hand, fruit set would be expected to be higher than in mass-flowering species. In *Anemopaegma laeve*, however, the relatively low fruit set (8.3%) was related to the strong negative impact of a seed predated weevil (Coleoptera: Curculionidae) and a flower-damaging stingless bee (*Trigona spinipes*) (Carvalho et al., 2006; see also Milet-Pinheiro and Schlindwein, 2008). Arguments concerning the importance of attracting pollinators from relatively long distances to apparently super-abundant flowers (Dafni et al., 1997), providing a highly localized and dense array of resources that influence the foraging strategies of the pollinators (Kevan, 1990), and paternity selection by the plant (Willson, 1983) can all be invoked as parts of the plants' sexual reproductive strategies. For the four species, we studied the phenomenon of stigmatic closure and re-opening seems to have an explanation in the plants' sexual reproduction in which cross-pollination results in the stigmatic capture of only doses of cross-pollen delivered. Under high levels of geitonogamy, however, permanent stigma closure in Bignoniaceae should diminish fruit set.

Acknowledgements

We thank Vernon G. Thomas and Luís Primo for suggestions, IBAMA for the permission to work in the

National Park of Catimbau, Gabriela D. Bieber for help in statistical analysis and Reisla Oliveira for suggestions on the manuscript. This study was supported by grants of CAPES, CNPq and Fundação O Boticário de Proteção à Natureza.

References

- Andrade, K.V.S., Rodal, M.J.N., Lucena, M.F.A., Gomes, A.P.S., 2004. Composição florística de um trecho do Parque Nacional do Catimbau, Buíque, Pernambuco-Brasil. *Hoehnea* 31, 337–348.
- Bawa, K.S., 1974. Breeding systems of tree species of a lowland tropical community. *Evolution* 28, 85–92.
- Bertin, R.I., 1982. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). *Am. J. Bot.* 69, 122–134.
- Carvalho, A.T., Santos-Andrade, F.G., Schlindwein, C., 2006. Baixo sucesso reprodutivo de *Anemopaegma laeve* (Bignoniaceae) no Parque Nacional do Catimbau, Pernambuco. *Rev. Bras. Bioc.* 5, 102–104.
- Dafni, A., Lehrer, M., Kevan, P.G., 1997. Spatial flower parameters and insect spatial vision. *Biol. Rev.* 72, 239–282.
- Darwin, C., 1876. The effects of cross and self-fertilization in the vegetable kingdom. London.
- Frankie, G.W., Haber, W.A., Bawa, K.S., 1983. Characteristics and organization of the large bee pollination system in the Costa Rican dry forest. In: Jones, C.E., Little, R.J. (Eds.), *Handbook of Experimental Pollination Biology*. Van Nostrand Reinhold Comp., New York, pp. 411–448.
- Fetscher, A.E., 2001. Resolution of male-female conflict in an hermaphroditic flower. *Proc. R. Soc. London, Ser. B* 268, 525–529.
- Fetscher, A.E., Kohn, J.R., 1999. Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). *Am. J. Bot.* 86, 1130–1135.
- Fetscher, A.E., Rupert, S.M., Kohn, J.R., 2002. Hummingbird foraging position is altered by the touch sensitive stigma of bush monkeyflower. *Oecologia* 133, 551–558.
- Gentry, A.H., 1978. Anti-pollinators for mass-flowering plants? *Biotropica* 10, 68–69.
- Gentry, A.H., 1980. Bignoniaceae. *Flora Neotropica*, Monograph no. 25(I). The New York Botanical Garden.
- Gentry, A.H., 1990. Evolutionary patterns in neotropical Bignoniaceae. *Mem. NY Bot. Gard.* 55, 118–129.
- Gentry, A.H., 1992. Bignoniaceae. *Flora Neotropica*, Monograph no. 25(II). The New York Botanical Garden.
- Gomes, A.P.S., Rodal, M.J.N., Melo, A.L., 2006. Florística e fitogeografia da vegetação arbustiva subcaducifolia da Chapada de São José, Buíque, PE, Brasil. *Acta Bot. Bras.* 20, 37–48.
- Gottsberger, G., Silberbauer-Gottsberger, I., 2006. Life in the Cerrado: a South American Tropical Seasonal Ecosystem. Vol. II – Pollination and seed dispersal. Ulm, Reta Verlag.
- James, E.A., Knox, R.B., 1993. Reproductive biology of the Australian species of the genus *Pandorea* (Bignoniaceae). *Aust. J. Bot.* 41, 611–626.
- Kevan, P.G., 1990. How large bees, *Bombus* and *Xylocopa* (Apoidea: Hymenoptera), forage on trees. *Ethol. Ecol. Evol.* 2, 233–242.

- Milet-Pinheiro, P., Schlindwein, C., 2008. Pollination in *Jacaranda rugosa* (Bignoniaceae): euglossine pollinators, nectar robbers and low fruit set. *Plant Biol.* (in press).
- Morawetz, W., 1982. Morphologisch-ökologische Differenzierung, Biologie, Systematik und Evolution der netropischen Gattung *Jacaranda* (Bignoniaceae). Österreichische Akademie der Wissenschaften. Math.-nat.Klasse, Denkschriften.
- Newcombe, F.C., 1922. The significance of the behavior of sensitive stigmas. *Am. J. Bot.* 9, 99–120.
- Newcombe, F.C., 1924. The significance of the behavior of sensitive stigmas II. *Am. J. Bot.* 11, 85–93.
- Pinto, C.E., Oliveira, R., Schlindwein, C., 2008. Do consecutive flower visits within a crown diminish fruit set in mass-flowering *Hancornia speciosa* (Apocynaceae)? *Plant Biol.* 10, 408–412.
- Richardson, S.C., 2004. Benefits and costs of floral visitors to *Chilopsis linearis*: pollen deposition and stigma closure. *Oikos* 107, 363–375.
- Ritland, C., Ritland, K., 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex (Scrophulariaceae). *Am. J. Bot.* 76, 1731–1739.
- Rodal, M.J.N., Andrade, K.V.A., Sales, M.F., Gomes, A.P.S., 1998. Fitossociologia do componente lenhoso de um refúgio vegetacional no município de Buíque, Pernambuco. *Rev. Bras. Biol.* 58, 517–526.
- Singh, J., Chauhan, S.V.S., 1996. Morphological changes in the stigma of seasonally transient sterile *Tecoma stans* L. *Phytomorphology* 46, 1–7.
- Statsoft, 2001. Statistica (data analysis software system). version 6.0. <www.statsoft.com>. Tulsa, USA.
- Stephenson, A.G., Thomas, W.W., 1977. Diurnal and nocturnal pollination of *Catalpa speciosa* (Bignoniaceae). *Syst. Bot.* 2, 191–198.
- Stevens, A.D., 1994. Reproduktionsbiologie einiger Bignoniaceen im Cerrado Brasiliens. *Biosystematics and Ecology Series 5*, Österreichische Akademie der Wissenschaften.
- Sudene, 1990. Dados pluviométricos mensais do Nordeste. Estado de Pernambuco. *Pluviometria 6*. Superintendência do Desenvolvimento do Nordeste, Recife.
- Thieret, J.W., 1976. Floral biology of *Probooscidea lousianica* (Martyniaceae). *Rhodora* 78, 169–179.
- Webb, C.J., Lloyd, D.G., 1986. The avoidance on interference between the presentation of pollen and stigmas in angiosperms. *NZ J. Bot.* 24, 163–178.
- Willson, M.F., 1983. *Plant Reproductive Ecology*. Wiley-Interscience, New York, NY.
- Yang, S.X., Yang, C.F., Zhang, T., Wang, Q.F., 2004. A mechanism facilitates pollination due to stigma behavior in *Campsis radicans* (Bignoniaceae). *Acta Bot. Sin.* 46, 1071–1074.
- Zar, J.H., 1999. *Biostatistical Analysis*, fourth ed. Prentice Hall, New Jersey.