BIOTROPICA 37(3): 381–388 2005 10.1111/j.1744-7429.2005.00050.x

Limited Fruit Production in *Hancornia speciosa* (Apocynaceae) and Pollination by Nocturnal and Diurnal Insects[1]

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ABSTRACT

Frequency and efficiency of pollinator visits strongly influence the reproductive success of self-incompatible plants. We investigated the breeding and pollination systems of *Hancornia speciosa*, a small tree that produces fleshy berries used in the Brazilian fruit industry. Observation and experiments were carried out in Northeastern Brazil. Thirty-three species of the visitor were recorded. Hawkmoths (Sphingidae), bees (Euglossini and Centridini), and butterflies (Nymphalidae and Hesperiidae) with long mouth parts were effective pollinators of *H. speciosa.* Access to nectar, the only reward for flower visitors, is determined by corolla tube length. Nylon threads of various diameters and dried mouth parts from a number of flower visitors were used in experiments to simulate flower visits. The number of pollen grains removed during such simulated visits showed no significant difference. Although xenogamic, *H. speciosa* presented a low pollen/ovule ratio (77). This might be related to the high efficiency of its pollination mechanism. Flowers of *H. speciosa* had 76 ovules on average. Seed set varied from 1 to 25, indicating that individual flowers received different amounts of outcross-pollen. Fruit production of hand cross-pollinated flowers increased by 90 percent when compared to natural pollination, suggesting pollinator limitation of *H. speciosa*.

RESUMO

A frequência e a eficiência das visitas dos polinizadores influenciam fortemente no sucesso reprodutivo de plantas auto-incompatíveis. Investigamos o sistema reprodutivo e de polinização de *Hancornia speciosa*, uma arvoreta que produz bagas carnosas utilizadas comercialmente no Brasil. As observações e os experimentos foram realizados no Nordeste do Brasil. Foram registradas 33 espécies de visitantes. Esfingídeos (Sphingidae), abelhas (Euglossini e Centridini) e borboletas (Nymphalidae e Hesperiidae) com peças bucais longas foram os polinizadores efetivos de *H. speciosa*. O acesso ao néctar, único recurso oferecido aos visitantes florais, é determinado pelo comprimento do tubo da corola. Pedaços de náilon de vários diâmetros e peças bucais secas de vários visitantes florais foram utilizadas em experimentos para simular visitas às flores. O número de grãos de pólen removidos durante uma só visita não diferiu significativamente entre os vários tratamentos. Apesar de xenogâmica, *H. speciosa* apresenta baixa razão pólen/óvulo (77), o que deve relacionar-se à alta eficiência do mecanismo de polinização. Flores de *H. speciosa* têm 76 óvulos em média. Contudo, o número de sementes produzidas variou de 1 a 25, indicando que as flores receberam diferentes quantidades de pólen exógeno. A produção de frutos a partir da polinização cruzada aumentou 90 per cento em relação à polinização natural, sugerindo que o baixo número de polinizadores limitou a produção de frutos de *H. speciosa.*

Key words: Apocynaceae; Brazil; Euglossine; Hancornia speciosa; *Hesperiidae; Sphingidae, pollination; Tabuleiro Nordestino*.

FLOWERS OF THE PREDOMINANTLY TROPICAL APOCYNACEAE show a complex pollination mechanism favoring cross pollination (Fallen 1986). In most species, the style head is functionally divided into (1) a stigmatic receptive area at the base that receives pollen from mouth parts of flower visitors; (2) a medium secretory area that produces a sticky mucilaginous substance that glues to the visitors' tongue while retracting it after nectar collection; and (3) an apical nonreceptive section which receives self-pollen from the introrse anthers and frequently forms a pollen chamber together with the anthers (Schick 1980, 1982). Nectar, in general, is the only floral resource available (Fallen 1986, Galetto 1997).

Functional aspects of the Apocynaceae flower structure have been considered by several authors (*e.g.*, Rowley 1980; Schick 1980, 1982; Fallen 1986). Information on plant–pollinator relationships and field observations of flower visitors, however, are scarce (Alberts & van der Maesen 1994). Insects are the main floral visitors of Apocynaceae (Fallen 1986, Alberts & van der Maesen 1994, Endress 1994). The pollination mechanism in large apocynaceous flowers may have evolved to attract insects with long and strong mouth parts to penetrate the flowers and obtain nectar (Schick 1982, Lopes & Machado 1999). Hermaphrodite flowers are common and self-compatibility seems to be rare in the family (Rowley 1980). Many plant species depend on their pollinators to achieve high reproductive success. The behavior and abundance of pollen vectors are also important ecological factors that influence plant fitness (Ramsey 1995, Proctor *et al.* 1996).

Hancornia speciosa Gomez is a small tree that occurs from Northeast Brazil to Bolivia (Engler 1964, Lorenzi 1992). The species is a typical component of the Central Brazilian vegetation of the Cerrado and of the Tabuleiro Nordestino in Northeastern Brazil (Tavares 1964, Rizzini 1997). The plants are 2–5 m high and have white, nocturnal flowers with a long corolla tube and an apical platform, and produce a sweet scent, suggesting a sphingophilous flower syndrome (Müller 1873, Knuth 1898, Vogel 1954, Faegri & Van Der Pijl 1979). They produce fleshy berries locally known as "mangaba" (Barros 1968, 1970; Corrêa 1974). Regionally, the fruit possesses a high socioeconomic potential and is commercialized as mangaba juice or ice cream. Various regional research bodies have implemented studies on cultivation and genetic improvement of the species.

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There is no information on the breeding system, flower visitors, and effective pollinators of *Hancornia speciosa*. Considering the ecological and economical importance of *H. speciosa* in Northeastern and Central Brazil, we asked: (1) Who are the flower visitors? (2) Which are the effective pollinators? and (3) Given its breeding system, in what manner does *H. speciosa* depend on its pollinators to produce fruits?

METHODS

STUDY SITE.—The study was undertaken from December 1998 until September 2000, at the National Reserve Guaribas (Reserva Biológica Guaribas-IBAMA) at 06°44′32″S; 35°08′33″W, in the district of Mamanguape, Paraíba, Northeastern Brazil.

The study site has distinct dry and rainy seasons. The rainy season is from March to June and the dry season from September to December. Annual median temperatures oscillate between 24 and 26°C (IBAMA 1995).

The vegetation is a savanna, regionally called "Tabuleiro Nordestino," a disjuncted occurrence of Central Brazilian Cerrado with which it shares floristic and physiognomic patterns (Prates *et al.* 1981, Oliveira-Filho & Carvalho 1993). The Tabuleiro Nordestino occurs on poor, sandy soils and is characterized by scattered trees and shrubs surrounded by a diverse herbaceous layer. Among the trees and shrubs, elements of the Central Brazilian Cerrado vegetation include *Hancornia speciosa*, *Curatella americana* (Dilleniaceae), *Byrsonima cydoniifolia* (Malpighiaceae) and *Bowdichia virgilioides* (Fabaceae), intermingled with typical species of the Tabuleiro Nordestino like *Campomanesia dichotoma* (Myrtaceae), *Krameria tomentosa* (Krameriaceae), *Guettarda platipoda* (Rubiaceae), *Hirtella ciliata, H. racemosa* (Chrysobalanaceae), and *Ouratea fieldingiana* (Ochnaceae) (Andrade-Lima 1960, Tavares 1988a,b, Oliveira-Filho & Carvalho 1993).

FLORAL MORPHOLOGY.—Flowers ($N = 150$) from 15 trees were used to assess mean corolla length, diameter of flower tube entrance, length of style head, and diameter of the aperture, through which flower visitors insert their mouth parts. Flower tubes were measured with a ruler and further measurements were taken with a Leica MZ12 stereo-microscope equipped with an ocular micrometer.

POLLEN ANALYSIS.—We prepared pollen reference slides of *H. speciosa* and of other sphingophilous plant species occurring at the study site. The pollen preparations of each species were mounted in pure glycerin gelatin and in glycerin gelatin stained with basic fuchsin solution, covered with a cover glass and sealed with paraffin (Louveaux *et al.* 1978, Wittmann & Schlindwein 1995). The reference slides were stored in the pollen reference collection of the Botanical Department of the Federal University of Pernambuco, Recife (UFPE). Pollen grains adhering to the mouth parts of flower visitors of *H. speciosa* were prepared in the same way and identified by comparison with the pollen reference collection. Additional pollen preparations were made of mouth parts of hawkmoths, which were captured on black lights at the study site from March 1999 to January 2000 (Darrault & Schlindwein 2002). SEM photos were taken with a Zeiss DSM 940.

FLOWER BIOLOGY, BREEDING SYSTEM, AND EFFECTIVE POLLINATORS.—Anthesis was determined by monitoring 48 marked flowers in 30-min intervals until they dehisced. Five trees were isolated in tulle cages to prevent flower visits. The flowers of these plants were used to measure nectar production and to test the breeding system. The volume of nectar accumulated in five flowers was measured during anthesis with a micro-syringe (Hamilton 10 ml). The sugar concentration was measured with a portable refractometer (Atago, Tokyo, Japan). To determine the concentration of the small amounts of nectar, the measuring surface of the refractometer was covered with a small piece (about 1 cm^2) of acrylic (J. Nuñez, pers. comm.).

To determine the breeding system of *H. speciosa*, three treatments were conducted on 40 flowers of each of the five bagged trees: hand cross-pollination, hand self-pollination, and spontaneous self-pollination without manipulation. The marked flowers of the five non-bagged trees that were exposed to flower visitors served as controls. Only one flower per branch was marked, to avoid possible competition effects among flowers on the same branch (Niesenbaum 1996). Each manually pollinated flower and pollen donor (in the case of cross pollination) were manipulated with a piece of nylon thread, simulating a flower visit. The nylon thread was introduced into the flower tube only once in the test for cross pollination and three times in the test of hand self-pollination.

Developing fruits were counted weekly during the first month and at 10-d intervals, until maturity. The seeds of the mature fruits were counted. *T*-tests were used to compare hand pollination and controls and one-way ANOVA to compare hand cross-pollination, hand self-pollination, and controls (Zar 1996).

The breeding system of *H. speciosa* was also characterized by the pollen/ovule ratio (P/O; Cruden 1977). The P/O ratio was determined by counting pollen grains and ovules of 24 flowers. One anther from each flower was removed and submerged in a solution of basic fuchsine 0.1 percent in ethanol 70 percent and transferred to a decantation box (capacity 1 ml), where the pollen grains were removed and counted with an inverted microscope. The total amount of pollen per flower was obtained by multiplying the number of pollen grains per anther by the number of anthers. Ovules were counted with a stereo-microscope (Leica MZ12).

FLOWER VISITORS.—Diurnal and nocturnal flower visitors were collected from *Hancornia speciosa* flowers, recording the time of visits. We also collected sphingids attracted to a blacklight trap, a white sheet extended between posts at the study sites. The hawkmoths were collected during eight nights, from 1800 to 0600 h in the blooming season. The pollen grains attached to the mouth parts of the sphingids were identified by comparison with the pollen reference collection of the working group. The specimens were stored in the entomological collections of the Department of Systematics and Ecology of the Federal University of Paraíba, João Pessoa (UFPB) and the Federal University of Pernambuco, Recife (UFPE).

EVALUATION OF POLLINATORS.—To test for a possible relation between the thickness of the proboscis of the different groups of flower visitors and the number of pollen grains removed at each visit, the following experiment was carried out: pieces of nylon thread with diameters of 0.20, 0.30, 0.40 and 0.80 mm, imitating the thickness of mouth parts of the various flower visitors, were inserted into a fresh, not visited flower of *H. speciosa* and the adhering pollen grains were extracted and counted. For each diameter, 10 replications were used. Differences among the number of extracted pollen grains were determined using the Kruskal–Wallis test (Zar 1996).

RESULTS

FLOWER MORPHOLOGY.—The flowers of *H. speciosa* are hypocrateriform, presenting a long (mean = 3.4 cm; range = 2.5–4.2) and narrow (mean diameter 0.12 cm) flower tube (Fig. 1). The anthers are located around the apex of the style head, and are not fused to it. The flowers show secondary pollen presentation: the anthers show introrse dehiscence and, before the onset of anthesis, shed their pollen grains onto the apex of the style head, forming a pollen chamber (Fig. 1 a,b).

The mouth parts of the flower visitors pass through apertures (mean = 1.0 mm) between the filaments. They extend to form channels that are delimited by five rows of long stiff hairs, which continue downward from the place of insertion of the filaments, and further five rows of shorter, finer hairs which intercalate with them. They guide the mouth parts of the flower visitor toward the nectaries at base of the corolla. (Fig. 1 c,d).

The style head is about 2.1 mm long and shows three sections (Fig. 1e): (1) an apical bilobed portion, where pollen is deposited, (2) a medium thinner portion, which is covered by a sticky mucilage, and (3) an inferior pollen receptor portion with a slightly concave surface which is located under a circle of thick hairs directed downward.

The ovary is located at the base of the corolla tube, and contains 76 ovules on average ($N = 24$; range = 52–81). One flower produces, on average, 5877 tetra (-penta)-zonocolporate, psilate, spherical pollen grains ($N = 25$; range = 3705–9045). The average P/O ratio was 77.3 ($N = 24$).

ANTHESIS AND PHENOLOGY.—Individuals of *H. speciosa* flowered from October to January with a peak in November/December and produced mature fruits from January to April. Anthesis started between 1530 and 1630 h and continued up to 1000 h of the following day. Monitoring of 48 marked flowers revealed synchronized opening and closing. When completely opened, about 90 min after anthesis, the flowers already contained nectar. Sugar concentration varied from 15.6 to 23.1 percent. Mean nectar volume was 3.6 μl. The sweet flower scent intensified at dusk and remained intense throughout the night, and had disappeared by morning.

BREEDING SYSTEM.—The pollination experiment showed that *H. speciosa* is self-incompatible. All manually pollinated flowers initiated fruit development, but except for one fruit with a single seed,

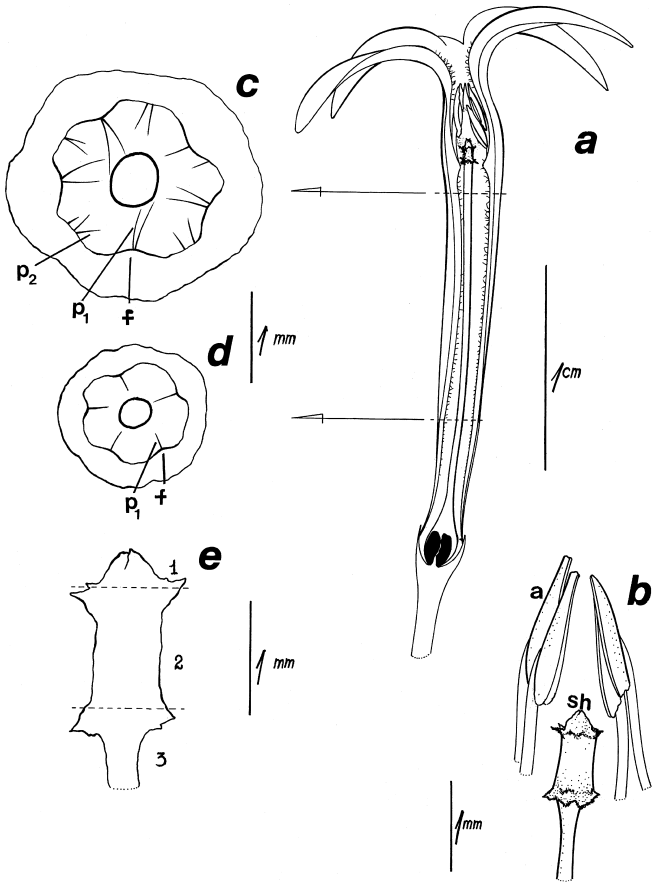

FIGURE 1. Flower of *Hancornia speciosa*. (a) Longitudinal section; (b) detail of style head and anthers. Anthers dehisce introrse and deposit pollen onto the style head, forming a pollen chamber; (c,d) transverse sections: (c) just beneath style head, (d) at the inferior third of the flower tube. Longitudinal rows of hairs following filaments form channels that guide flower visitors' mouth parts. The intercalated rows of short hairs are absent at the inferior half of the flower tube; (e) scheme of the style head: 1: apical part, base of the pollen chamber, 2: secretory region, covered with a mucilaginous, adhesive secretion, 3: inferior hair ring delimiting the receptive region. a = anthers; sh = style head; f = filaments; p_1 = hair rows following the insertion of the filaments; p_2 = intercalated hair rows.

all fruits resulting from hand self-pollination were aborted (Table 1). There was a significant difference among the number of remaining fruits (ANOVA, df = 2, $F = 5.923$; $P < 0.05$). The curves with the rates of fruit loss in different treatments show different rates of change, indicating accelerated abortion of immature fruits after hand and after spontaneous self-pollination.

Seed set per fruit was, on average, 4 times larger for manual cross-pollination when compared with natural pollinated controls. On average, the fruits resulting from hand cross-pollination showed 12 percent fertilized ovules, those of open pollinated flowers 3 percent and those of hand self-pollination 1 percent.

TABLE 1. *Number of fruits and seeds of* Hancornia speciosa *showing different pollination modes.* H. speciosa *is self-incompatible. Fruit set of hand cross-pollinated flowers is significantly higher than of natural pollinated flowers ($t = 2.262$; $g = 86$; $P < 0.05$).*

	Hand self-pollination	Hand cross-pollination	Natural pollination	Spontaneous self-pollination
N	40	43	45	40
Fruit set	1	9	5	0
Total seed number	1	80	10	0
Average seed number per fruit ± SD	1.0	8.8 ± 6.6	2.0 ± 0.7	0

FLOWER VISITORS.—We recorded 77 individuals of 33 insect species as flower visitors of *H. speciosa* (Table 2): 11 species of bee (Hymenoptera) (32%) and 23 of Lepidoptera. Among the latter, 52 percent were species of Sphingidae, 39 percent of Hesperiidae, and 9 percent of Nymphalidae.

Diurnal flower visitors were mainly euglossine bees, *Heliconius* butterflies and Hesperiidae butterflies. All hawkmoths were recorded at night. Individuals of *Aellopos fadus* (Sphingidae) visited *Hancornia* flowers both at night and in the morning (0800 h). All insects visited the flowers of *H. speciosa* to collect nectar. Pollen grains adhered exclusively to their mouth parts since only these could be inserted into the flower tube. Generally, the flower visitors collected nectar in various flowers of a single *H. speciosa* plant and then continued foraging in flowers of other individuals of the species. Specimens of *Eulaema nigrita* and *E. bombiformis* visited flowers primarily at the top of the crown.

Females of *Xylocopa frontalis* were nectar thieves, perforating the base of the flower tube to collect nectar. Workers of a stingless honey bee *Trigona* sp. (Apidae, Meliponini) were observed to bite fruits and flower stalks while collecting latex. Most of the flower visitors had long mouth parts. Among the 21 species whose proboscis was measured, 12 had mouth parts long enough to reach the bottom of medium-sized flower tubes of *H. speciosa* (3.4 cm), 3 had mouth parts just long enough to reach nectar in short flower tubes and 6 had mouth parts too short to reach the base of the flower tubes, even in flowers with the shortest flower tubes (Fig. 2).

Pollen grains were found on the mouth parts of species of all groups of visitors (Table 2; Fig. 3). Pollen analysis revealed that *H. speciosa* shared flower visitors with at least 32 species of plants at the study site (*e.g.*, Boraginaceae- *Cordia*; Convolvulaceae- *Ipomoea*; Mimosaceae- *Calliandra*, *Inga*; Myrtaceae; Rubiaceae- *Guettarda*; Tiliaceae- *Luehea*). More than two thirds of the flower visitors of *H. speciosa*, hawkmoths and butterflies, also visited flowers of *Guettarda platipoda* (Rubiaceae).

POLLINATION MECHANISM.—Together with the anthers, the style head forms a pollination apparatus that favors cross pollination. While looking for nectar, the flower visitor inserts its proboscis into the flower tube up to the base of the corolla. During insertion,

TABLE 2. *Flower visitors of* H. speciosa *recorded at the National Reserve Guaribas, Paraíba, Northeastern Brazil.*

Flower visitors	Number of individuals	Presence of pollen of *H. speciosa*
HYMENOPTERA		
APIDAE – ANTOPHORINAE		
Centris sp.	2	No
Epicharis (Xanthemisia) bicolor Lepeletier, 1841	1	No
XYLOCOPINAE		
Xylocopa (*Megaxylocopa*) *frontalis* (Olivier, 1789)	2[a]	–
EUGLOSSINAE		
Euglossa sp	1[a]	–
Eulaema bombiformis (Packard 1869)	3	Yes
Eulaema cingulata (Fabricius, 1804)	3	Yes
Eulaema flavescens (Friese 1899)	1	No
Eulaema nigrita Lepeletier 1841	7	Yes
Exaerete smaragdina Guérin, 1845	1	No
BOMBINAE		
Bombus brevivillus Franklin, 1913	1	
LEPIDOPTERA		
HESPERIIDAE – HESPERIINAE		
Perichares philetes adela	3	Yes
URBANINAE		
Bugalotis sp.	1	Yes
Dysocephaly nicephorces (Hewiston, 1867)	1	Yes
Historis acrouta	1	Yes
Nascus phocus (Cramer, 1777)	2	Yes
Phocides pigmaliaes	1	Yes
Urbanus durantes durantes (Stoll, 1790)	2	Yes
Urbanus proteus proteus (Linnaeus, 1758)	2	Yes
Urbanus teleus	1	Yes
NYMPHALIDAE		
Heliconius phyllis (Fabricius, 1793)	1	Yes
Heliconius nanna Stichel, 1899	12	Yes
SPHINGIDAE – MACROGLOSSINAE		
Hyles euphorbiarum (Guérin-Méneville & Percheron, 1835)	2	Yes
Aellopos fadus (Crarmer. 1775)	1[b]	Yes
Agrius cingulatus (Fabricius, 1775)	2	Yes
Enyo ocypete (Linnaeus , 1758)	1[b]	Yes
Erinnyis ello (Linnaeus, 1758)	5	Yes
Isognathus caricae (Linnaeus, 1758)	1[b]	Yes
Isognathus menechus (Boisduval, [1875])	4	Yes
Pachylia ficus Linnaeus, 1758	2	Yes
Pachylia syces (Hübner, [1819])	1[b]	Yes
SPHINGINAE		
Manduca difissa (Butler, 1871)	1[b]	Yes
Manduca sexta paphus (Cramer, 1779)	6	Yes
Neogene dyanaeus (Hübner, [1827]–[1831])	2[b]	Yes
Total number of individuals	77	

[a]Individuals observed, not collected.

[b]Individuals attracted to blacklight and mixed light.

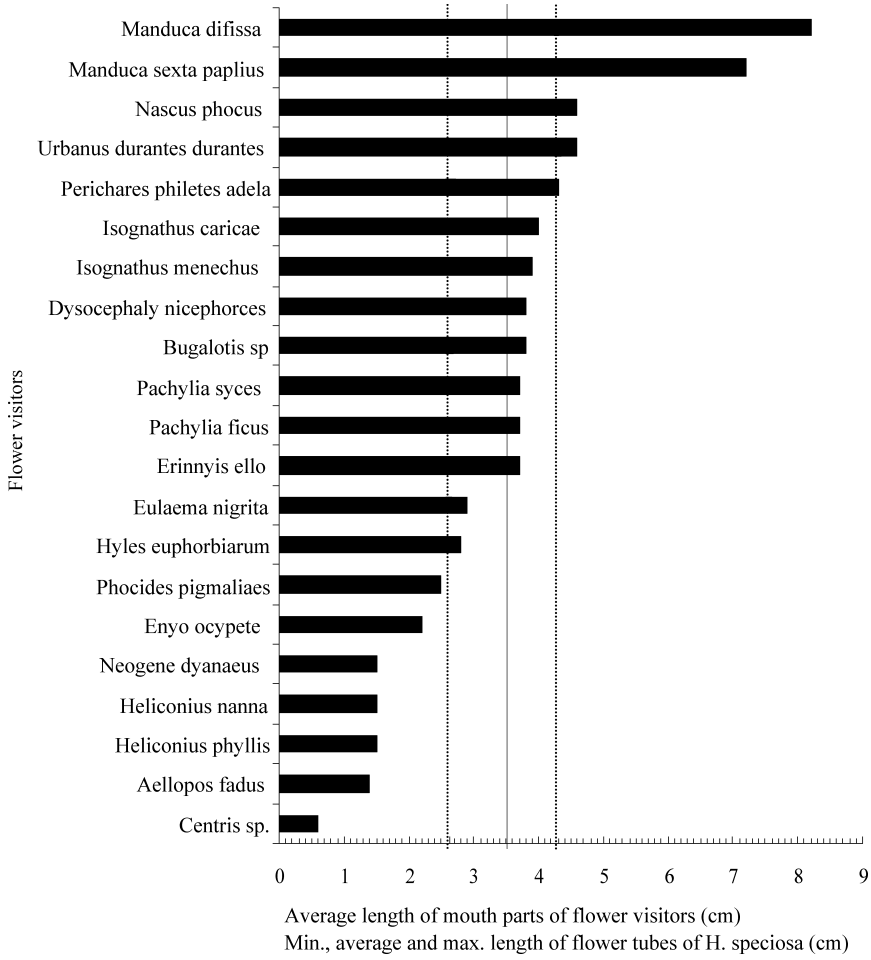

FIGURE 2. Average length of mouth parts of flower visitors of *H. speciosa* at the Reserva Biológica Guaribas. Vertical lines represent minimum (2.5 cm), average (3.4 cm), and maximum (4.2 cm) length of the flower tubes of *H. speciosa.*

the mouth parts do not come in contact with the pollen chamber. After experimentally introducing a piece of a nylon thread into the flower tube, half of the tube was removed to check, with a hand lens, for pollen on the inserted part of the nylon thread. No pollen grains were recorded ($N = 10$). When removed, the proboscis scrapes the hair ring at the base of the style head, and outcross-pollen is deposited onto the receptive area of the stigma. The proboscis then passes the medium portion of the style head where it is covered with an adhesive substance. As the entrance of the corolla tube is constricted, the proboscis is directed to the center of the tube, passing the pollen chamber and removing pollen grains (Fig. 1a).

EFFECTIVE POLLINATORS.—The width of the base of the mouth parts of the flower visitors ranged from 0.4 mm in *Perichares philetes adela* to 1.0 mm in *Agrius cingulatus*. Most of the species of hawkmoths possessed thicker mouth parts (0.6–1.0 mm) than the representatives of *Heliconius* (0.43 mm), bees (0.6–0.9 mm), and Hesperiidae (0.4–0.5 mm).

Simulating flower visits with nylon threads of different diameters also demonstrated a high variation in the amount of pollen removed by threads of the same diameter (Fig. 4). Differences among the average number of grains removed by threads of different diameters, however, were significant (Kruskal–Wallis = 8.278; df = 3; $P < 0.05$). Maximum pollen removed with threads of 0.20 mm diameter (750 grains) was almost half that removed by thicker threads (0.3, 0.4, and 0.8 mm—1396, 1566, and 2066 pollen grains, respectively; Fig. 4).

DISCUSSION

FLOWER MORPHOLOGY.—The morpho-functional division of the style head of the flowers of *H. speciosa* observed in this study coincides with that described for the apocynaceous tribe Willughbeeae (Endress & Bruyns 2000). Until the mid-1990s this species was placed in the primitive tribe Carisseae of the subfamily Plumerioideae (Fallen 1986, Alberts & Maesen 1994). In this tribe, in

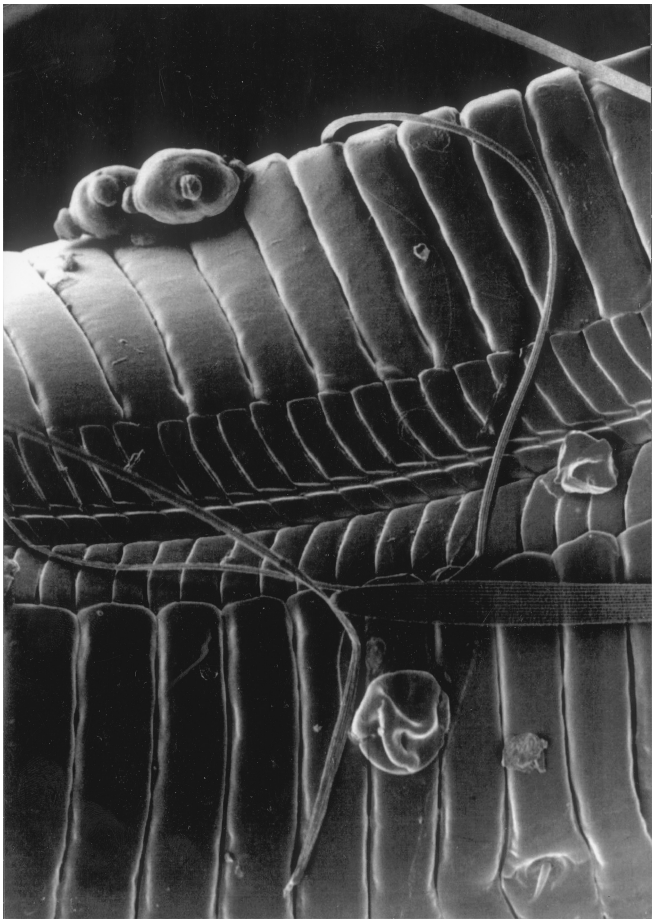

FIGURE 3. SEM photo of a pollen grain of *H. speciosa* adhering to the ventral face of *Isognathus menechus* mouth parts.

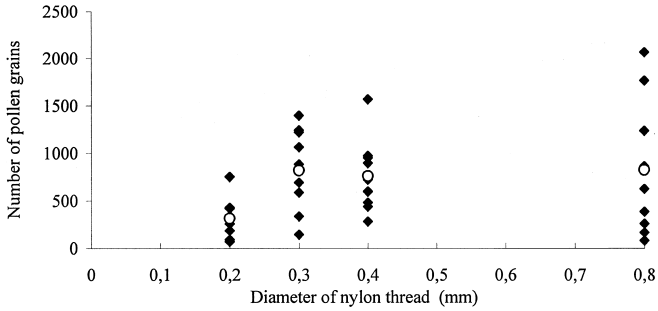

FIGURE 4. Number of pollen grains removed from flowers of *H. speciosa*, using nylon threads of different diameters (0.2, 0.3, 0.4, and 0.8 mm). The threads were inserted into fresh flowers, which had not been visited before. Using threads of 0.2 mm, the average quantity of removed pollen was less than half of that using thicker threads. Open circles represent the average number of pollen grained removed by threads of the different diameters.

contrast to Willughbeeae, the style head is scarcely differentiated, showing no inferior hair ring and the surface is uniformly receptive (Endress & Bruyns 2000).

REPRODUCTIVE SYSTEM.—Although results of the pollination experiments indicate that *H. speciosa* is an obligate xenogamous species, its pollen–ovule ratio classifies the species as facultative autogamous (Cruden 1977). *H. speciosa* presents an extraordinary low P/O ratio when compared to other xenogamic species. *Nerium oleander*, a further representative of Apocynaceae, also shows an extraordinarily low P/O ratio (Cruden 1977).

In Apocynaceae, the arrangement of the floral parts frequently includes structures to capture outcross- and to store self-pollen (Fallen 1986, Alberts & van der Maesen 1994). Hence, in a single visit, the floral apparatus removes the pollen which adhered to the visitor's mouth parts and then transfers a large amount of self-pollen to it. The result is a highly efficient pollination mechanism. Simulation of flower visits with nylon threads to fresh flowers of *H. speciosa* demonstrates the efficiency of this mechanism. The pollination apparatus of *H. speciosa* favors the removal of a great amount of pollen per visit. Moreover, experiments with cleaned, stretched, and dried mouth parts of flower visitors inserted into the corolla tubes resulted also in an efficient pollen removal. Mouth parts of *Eulaema nigrita* (Euglossini) removed up to 1262 pollen grains, *Heliconius nanna* (Nymphalidae) 1004, *Perichares philetes adela* (Hesperiidae) 614, and *Erinnys ello* (Sphingidae) 1994 pollen grains. Thus, visitors can remove up to 34 percent of the total pollen of a flower in one visit. This quantity would be enough to pollinate the ovules of 26 *Hancornia* flowers. The precise, optimized pollination mechanism of *H. speciosa* seems to allow a low pollen–ovule ratio for this xenogamous species. It may also be interpreted as a mechanism to economize pollen, a probably widespread feature in Apocynaceae (*sensu stricto*).

The amount of pollen removed in a single visit corresponds to the removal of one to two pollinia of a pentamerous flower of an Asclepiadaceae. Recently, several authors have suggested inclusion of the Asclepiadaceae in the family Apocynaceae (see references in Endress & Bruyns 2000). In this context, the removal of great amounts of pollen during single flower visits would seem to be part of the general trend of pollen presentation in small packets, which is common in Apocynaceae–Asclepiadaceae.

In *H. speciosa*, the transfer of pollen from the anthers to the apical portion of the style head results in secondary pollen presentation (Yeo 1993). The pollen grains always remain hidden from flower visitors in the pollen chamber inside the flower tube, however, different from most examples of plants with secondary pollen presentation. This floral mechanism may be (1) to protect pollen against pollen thieves; (2) to deposit pollen onto safe parts of the flower visitors to ensure little pollen loss on the vector; and (3) to free pollen in distinct packets. All plant species with secondary pollen presentation treated in Cruden (1977) show a P/O ratio below the average for xenogamous species.

The pollination mechanism in *H. speciosa* increases pollination efficiency because it (1) reduces pollen loss; (2) separates the place of pollen presentation from the receptive stigmatic surface; and (3) guarantees that during a flower visit the flower first receives outcross-pollen and then donates self-pollen. All these features make

autogamy difficult and favor cross pollination, but do not prevent geitonogamy.

FLOWER VISITORS AND POLLINATION.—Despite specialization by some flower visitors, flowers often attract several pollinator groups (Baker & Hurd 1968, Janzen 1980, Waser *et al.* 1996, Fleming *et al.* 2001). The flowers of *H. speciosa* attract nocturnal and diurnal flower visitors of various species, mainly Lepidoptera. We did not detect any species-specific relationship between the plant and its flower visitors. In all pollinator groups, some individuals possessed mixed loads of pollen from *Hancornia* and from other species on their mouth parts. All flower visitors, however, are long-tongued insects and contribute to pollination of *H. speciosa*. Hawkmoths seem, nevertheless, to be the most important pollinators. Their recorded flower visits are under-represented in our study, because numerous sphingid flower visitors were only observed and could not be caught at night.

To self-pollinate a flower, the flower visitor would have to insert its mouth parts into the same flower at least twice: first to remove self-pollen and during the second insertion to deposit it onto the style head. Two consecutive insertions of an individual on the same flower were never observed.

The length of the mouth parts of the flower visitors determines which insects reach the base of the flower tube to collect nectar. More than half of the flower-visiting species of *H. speciosa*, including bees, Hesperiidae, and Sphingidae, possess a proboscis long enough to reach the bottom of the flower tube. It is surprising that one-third of the flower-visiting species have mouth parts of insufficient length to reach the nectaries, even in the shortest flowers of *H. speciosa*. Flower visitors that can only reach the base of those flowers with shorter-than-average flower tubes (24 of the 125 measured flowers) would frequently not receive nectar rewards.

While looking for nectar in the flowers of *H. speciosa*, all flower visitors with mouth parts longer than 0.8 cm (distance from the base of the style head to the apex of the flower tube) touch the pollen and the receptive portion of the style head, and, therefore, may pollinate the flowers, even if they do not reach the nectary at the base of the flower tube. This explains the presence of pollen grains of *H. speciosa* on the proboscis of visitors with short mouth parts like *Aellopos fadus, Enyo ocypete, Neogene dinaeus, Heliconius phyllis*, and *H. nanna*.

The large variation in the number of seeds contained in mangaba fruits is probably related to the variable number of pollen grains deposited on the receptive surface of the style head. This would indicate that some flowers receive insufficient outcross-pollen. Observations of the foraging behavior of flower visitors revealed that representatives of all species visited several flowers of the same plant individual before visiting a second individual of *H. speciosa*. Due to the efficient scraping of pollen at the hair ring at the base of the style head, these successive visits to flowers of the same plant may result in the deposition of smaller amounts of outcross-pollen on the receptive stigmatic surface. Consequently, transfer rates of self-pollen will increase with each subsequent visit to the same individual flower. The ideal pollinator of *H. speciosa* would visit only one or a few flowers of the same tree and soon switch to flowers of another *Hancornia* plant.

LIMITATIONS TO FRUIT PRODUCTION OF *HANCORNIA SPECIOSA*.—Higher fruit production by hand cross-pollination when compared to natural pollination suggests that low pollinator numbers limited fruit production. We measured a difference from 11 percent in the fruit set for open pollinated flowers to 21 percent for hand cross-pollinated flowers, which led to an increase of 90 percent in fruit production. Comparing the seed set, the discrepancy is even more striking: hand cross-pollinated flowers produce, on an average, four times more seeds than open pollinated flowers. As seed number is positively correlated with fruit weight, fruit productivity of mangaba trees could be considerably increased by higher population densities of pollinators.

In the Tabuleiro Paraibano, there are generally two flowering periods of *H. speciosa* (Aguiar Filho & Bosco 1998). Fruit set during each flowering period may vary with oscillations in pollinator richness and abundance, especially of hawkmoths. The sphingofauna of the Tabuleiro Nordestino shows high seasonal variation in species number and abundance. Some species only appear during the dry season while others are restricted to the rainy season (Darrault & Schlindwein 2002). Our pollination study was made during the dry season. Different results in fruit set may appear during rainy season.

During our study, *H. speciosa* shared flower visitors particularly with *Guettarda platipoda* (Rubiaceae), a sphingophilous mass-flowering shrub (Darrault & Schlindwein 2002). Pollinator sharing may have a negative impact on the reproductive success of co-occuring plant species (Levin & Anderson 1970, Kephart 1983, Armbruster 1986). On an average, only 20 percent of the flowers of *H. speciosa* produce mature fruits (Aguiar Filho & Bosco 1998). The development of minute fruits with only one or a few seeds emphasizes the pollinator limitation in *H. speciosa* at the study site.

ACKNOWLEDGMENTS

We thank the mangabeira specialists Severino Aguiar-Filho and João Bosco (EMEPA, João Pessoa) for incentive support, Marcelo Marcelino and the team of the Reserva Biológica Guaribas (IBAMA), Sr. Sadam, Sr. Manuel Pedro, Sr. Oscar and Sr. Luís, for logistic support, Olaf Mielke (UFPR, Curitiba) for his help in the identification of Sphingidae and Hesperiidae, Isabel C. Machado (UFPE) and Celso F. Martins (UFPB) for discussion, Márcio M. L. Oliveira (UFAC) for the identification of the Euglossini, Roberto Sassi (UFPB) and Marcelo Guerra (UFPE) for the use of microscopes, Hans-Peter Stika and Anne Heller (University of Hohenheim, Germany) for help with SEM work, Rainer Wirth (University of Kaiserslautern, Germany) and Inara Leal (UFPE) for critical reading of the manuscript, Bob Allkin (Kew, London) for improving the English and Marcos S. Barbeitos, Cristina Buitrón, and Bruno Xavier for help in the field. This study received financial support from CAPES, CNPq and the A.F.W. Schimper Foundation.

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