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# Phenology and pollination biology of *Ceiba pentandra* (Bombacaceae) in the wet forest of south-eastern Costa Rica

## Fenología y biología de la polinización de *Ceiba pentandra* (Bombacaceae) en el bosque húmedo del Sudeste de Costa Rica

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**Abstract**: Phenological patterns and pollination biology of *Ceiba pentandra* were studied in natural populations located in wet forests of SE Costa Rica. Although the timing of leaf fall, flowering, and fruiting activity were restricted to the dry season and occurred in a small time period, this species showed great variability at temporal and individual level in the frequency and intensity of flowering and fruiting events. We observed high reproductive activity for wet forest trees in 2003 that coincided with an advance in flowering timing to December. Pollen of this species demonstrated high germination rates until 14 hours after grains were removed from flowers and in self-pollinated flowers, pollen tubes were able to grow over the style in a similar proportion to flowers with outcrossing pollination. However, in flowers with self-pollination, fruit set was nil and this result suggests the existence of late incompatibility barriers for this species. In addition, differences in fruit production for flowers with cross-pollination and natural pollination treatments suggest that fruit set in *C. pentandra* is partially limited for the arrival of exogenous pollen. In the wet forest of the SE of Costa Rica, *C. pentandra* flowers are visited by nectarivorous, frugivorous and generalist bat species. However, differences in diversity and abundance of floral visitors were observed in regards to other forest types (i. e., dry forest). Indeed, in this region, species such as *Phyllostomus discolor*, which is very abundant in Guanacaste, were not observed, and neither were a great number of bat species flying around flowering trees as is common for trees of this species located in the dry forests of Costa Rica.

**Key words**: Bombacaceae, *Ceiba pentandra*, Costa Rica, plant reproduction, phenology, pollination biology, bats.

**Resumen**: La fenología y diversos aspectos de la biología de la polinización *Ceiba pentandra* fueron estudiados en poblaciones en el bosque húmedo del SE de Costa Rica. Aunque la caída de hojas, floración y fructificación ocurren en un período limitado de tiempo en la época seca, existe una amplia variación temporal e individual en la frecuencia de floración y fructificación. Se observó un año (2003) de alta reproducción poblacional que coincide con un adelanto de la floración al mes de Diciembre. El polen de *Ceiba pentandra* muestra alta capacidad de germinación hasta 14 horas después de su remoción, y en flores autofecundadas es capaz de desarrollarse hasta el final del estilo en la misma proporción que en cruces outcross. Sin embargo el éxito de frutos self es nulo, lo que muestra barreras tardías de incompatibilidad. El incremento del fruit set con polinización cruzada con respecto a polinización natural muestra que *C. pentandra* es una especie limitada por polen exógeno. Las flores de *C. pentandra* del bosque húmedo del SE de Costa Rica son visitadas por varias especies de murciélagos, pero no fué observada la especie *P. discolor* que es muy abundante en Guanacaste. Tampoco se observaron grandes concentraciones de murciélagos como es común en el bosque seco de Costa Rica.

**Palabras clave**: Bombacaceae, *Ceiba pentandra*, Costa Rica, reproducción de plantas, fenología, biología de la polinización, murciélagos.

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**Fig. 1**: *Ceiba pentandra* trees at La Palma, Osa Peninsula. A flowering tree without leaves (left) is observed beside a tree in full leaf (right) with recently replaced foliage. This tree did not produce flowers that year. This scene is an example of the variation in the reproduction frequency among trees for this species.

## Introduction

*Ceiba pentandra* is a pantropical canopy tree of tropical dry and wet forests, distributed in the neotropics from Mexico to the Amazonian basin and in the Palaeotropics throughout western Africa (HARTSHORN 1983). This species is a tall (> 40 m) emergent deciduous tree that may reach a diameter (d.b.h.) of more than 200 cm. C. *pentandra* has hermaphroditic flowers with five stamens around a protruding style, and pink petals (CASCANTE-MARÍN 1997). Flowers show nocturnal anthesis and produce large amounts of nectar, and in neotropical forests phyllostomid bats are reported as pollinators (GRIBEL et al. 1999). Fruits are elliptic, with seeds surrounded by a pale yellow silk cotton, used for wind dispersal.

The flowering and fruiting episodes of C. *pentandra* are limited to the dry season in neotropical forests (FRANKIE et al. 1974, GRIBEL et al. 1999). For instance, on the Pacific Coast of Central America, trees located at different latitudes within this region flower simultaneously in January (LOBO et al. 2003). In the neotropics, the reproductive phenology of this species has been described as supra-annual with great variation in reproductive intensity between individuals, populations and years (FRANKIE et al. 1974, GRIBEL et al. 1999, LOBO et al. 2003). However, synchronised population patterns have been observed in years of greater reproductive intensity (GRIBEL et al. 1999, LOBO et al. 2003). This species practises mixed mating (MURAWSKI & HAMRICK 1992, LOBO et al. 2005) and self-pollen tubes grow normally in the styles (GRIBEL et al. 1999). However, manual self-pollinations in trees from the Amazonian basin results in low levels of fruit set (GRIBEL et al. 1999).

In the SE of Costa Rica, C. *pentandra* is an emergent tree with tall individuals (> 60 m) that are part of the landscape in lowland open areas, as well as in mountainous areas (Figure 1). Although many individuals remain in agricultural areas mainly due to the low value of its wood and because they are used to provide shade for livestock; the reproductive population size is gradually reduced by natural mortality, selective logging and constraints on natural regeneration (ROJAS-SANDOVAL 2004).

The main goal of this study was to provide data on phenological patterns of *Ceiba pentandra* populations located in wet forests of the SE Pacific Coast of Costa Rica, as well as general aspect on pollination biology mainly related to the capacity of this species to auto-fertilise and their dependence on pollinator agents. Specifically we present information on: (1) flowering and fruiting frequencies for C. *pentandra* populations located in SE Costa Rica, (2) the effect of pollen source (i. e., selfing vs. outcrossing) in the fruit set of trees located in these populations, (3) pollen viability, (4) differences in pollen loads and pollen tube numbers in flowers with self-pollination, outcross and natural pollination treatments, and (5) bat species visiting C. *pentandra* flowers in some locations of the SE forest of Costa Rica.

## Material and methods

### Study sites

We conducted our study in the tropical wet forest located in the South Pacific Coast of Costa Rica (8°32'N, 83°18'W). The dry season in this site extends from December to April but it is not as intense as in tropical dry forest. (GÒMEZ & HERRERA 1985) The average annual rainfall here is 3500 mm. All C. *pentandra* individuals used in this study were reproductive adults with a diameter at breast height greater than one metre. We identified two populations of isolated trees (Boruca-Chacarita n = 39 and La Palma-Puerto Jimenez n = 30) and two populations consisted of trees in continuous forest (Golfo Dulce Forestry Reserve n = 8, and Sirena Biological Station n = 16).

### Phenological observations

In order to determine the sequence of phenological phases of trees, we recorded the phenology of marked trees every two weeks from December to April for three years (2001, 2002 and 2003), starting on November 1st each year. Trees were observed with binoculars. Leaf, flower and fruit phenologies were determined based on the percentage of cover of the crown suggested by FOURNIER (1974).

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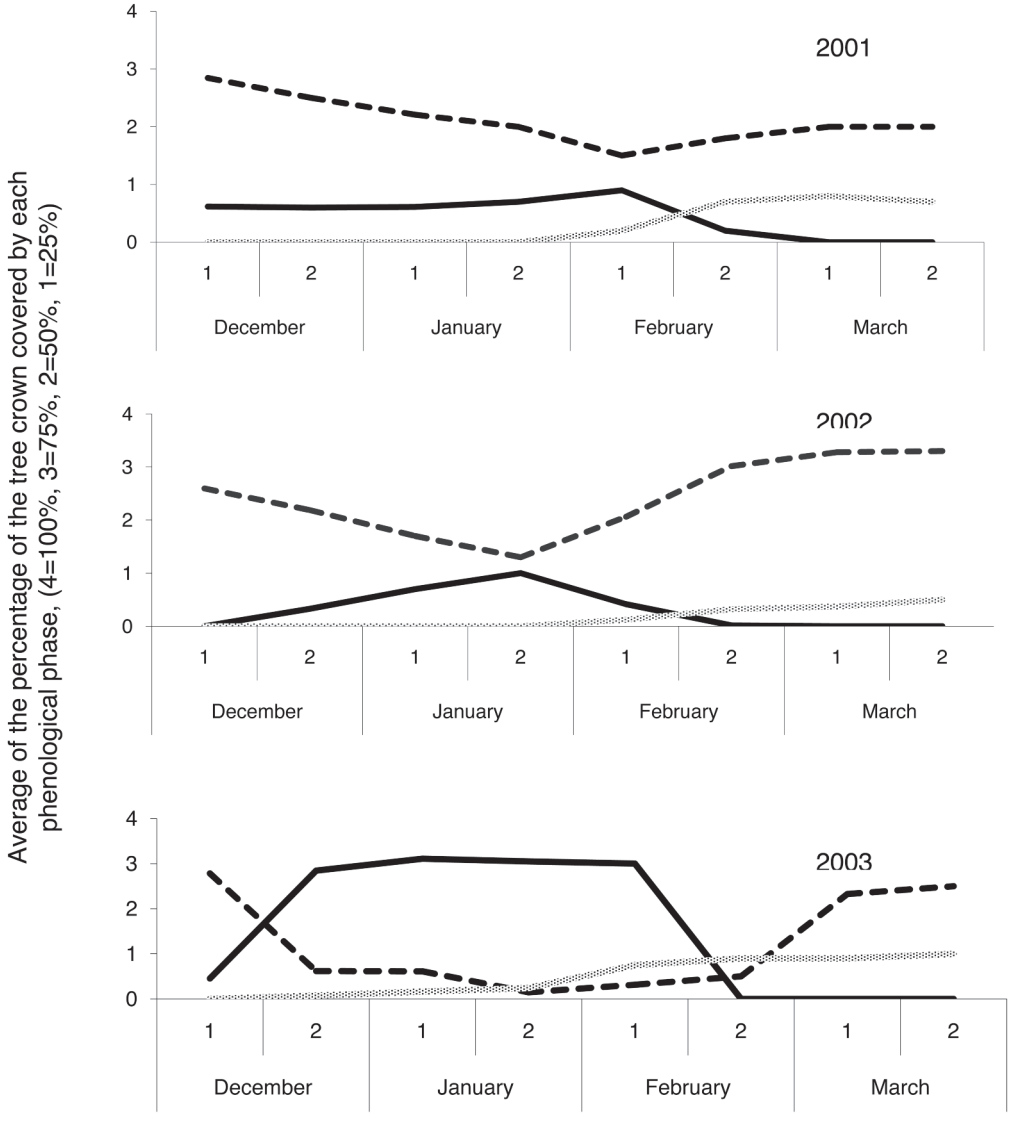


**Fig. 2**: Phenological patterns of leaves ..... flowers ——— and fruits ——— for *Ceiba pentandra* trees in the tropical wet forest of SE Costa Rica over three years (2001, 2002 and 2003).

## Pollen germination and pollen tube growth

Pollen germination experiments were performed following the methodology proposed by BREWBAKER & KWACK (1963). Closed flowers from two trees were bagged in late afternoon and pollen was removed immediately after flower anthesis and stored in Eppendorf tubes (0.5 ml) at room temperature. Pollen grains were placed in culture plates containing germination media and wet filter paper at 0, 1, 2, 14, 18, 22, 26, 36, 48, 60 and 72 hours after grains were removed from the flowers. Culture plates were kept at room temperature and the total of germinated pollen grains were counted under a light microscope after six hours. The presence of pollen tubes on stigma and style was analysed in flowers from six different trees, in which we performed pollination experiments (i. e. self-pollination, cross-pollination and natural pollination). Early in the morning, we removed stigma and style from flowers and preserved them in 70% ethanol. Once in the laboratory, stigmas and styles were stained with aniline blue and observed under a fluorescence microscope to determinate the total of germinated pollen grains on stigma area and pollen tubes growing over the style.

## Pollination experiments

To determine the breeding system of *C. pentandra*, two different pollination treatments (self-pollination and cross-pollination) were performed on 1158 flowers from five different trees. Flowers were marked and bagged before flower anthesis to prevent pollinator visitation. The treatments were: (1) Cross-pollination: when bagged flowers opened, they where emasculated and hand-pollinated with pollen from at least two to three different donors (n = 610). (2) Self-pollination: the bagged flowers were hand-pollinated with pollen from the same flower (n = 548); self and exogenous pollen were collected in plastic cups one hour after flower anthesis and hand pollinations were performed

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saturating the stigma with pollen (approximately 500 pollen grains per stigma) using a soft brush. After each treatment, marked flowers were bagged again and followed until fruit maturation or fruit abortion occurs. In addition, to analyse the reproductive success for this species under natural conditions, we selected and marked 798 flowers in 5 different branches in one *C. pentandra* tree. These flowers were monitored until fruit production or fruit abortion.

### Floral visitors

In order to determinate the diversity, abundance and activity of floral visitors to flowering *C. pentandra* trees, we placed two 12-meter mist nets in a natural corridor near a flowering tree. We performed this methodology for three different trees. Each captured bat was identified to species using the key of TIMM & LAVAL (1998). Pollen samples were collected from bodies and faces for all frugivorous and nectarivorous bat species captured using cubes of jelly stained with fucsine (BEATTIE 1971).

## Results

### Phenological patterns and reproductive success

All *C. pentandra* trees studied lost their leaves during the dry season, in December and January. Leaf fall activity reached its peak in the beginning of January. By the beginning of March, most trees had flushed large quantities of new leaves and by April, all trees were in full leaf (Fig. 2).

Flowering was also restricted to the dry season (Fig. 2). For the years 2001 and 2002, flowering activity started in the beginning of January. In 2003, however, trees bloomed prematurely and flowers were present on some trees in early December. The flowering peaks for 2001 and 2002 occurred at the end of January, but for 2003 occurred during the first two weeks of January (Fig. 3). This year most trees flowered profusely, but it was not followed by profuse fruiting. Seventy percent of the trees that bloomed that year aborted all the flowers. The duration of flowering was approximately 70 days. In regards to the fruiting period, in 2001 and 2002, the production of fruit began during the final two weeks of January, and fruiting peaked in March. For 2003, fruiting began in January as a result of early flowering activity (Fig. 2).

The percentage of individuals that flowered and fruited varied depending on the year. For instance, the lowest reproductive success was observed in 2002, when only 19% of the trees produced mature fruit. The greatest reproductive success was recorded in 2003, when 40% of trees produced mature fruit. In addition, irregularity in flowering and fruiting cycles was observed for individual trees. For 68 trees monitored over 3 years, it was possible to observe that 21% of trees never flowered, 39% flowered once, 28% flowered twice and 12% flowered during three consecutive years (Table 1). In addition, for the same 68 trees, 31% never produced mature fruit, 48% fruited one year, 18% fruited two years and only 3% were able to produce mature fruit during three consecutive years (Table 1).

In regards to reproductive success, profuse flowering episodes in many *C. pentandra* trees were followed by little or no fruit production. In these cases, the flowering process was followed by the abortion of flower buds and developing fruits. This phenomenon occurred both in isolated trees and trees located in areas of continuous natural forest. Total abortion of flower buds occurred in 15% of the population in 2001, 22% in 2002 and was most evident in 2003, when 40% of trees that flowered aborted all the flower buds.

Five hundred and fifty five flower buds and 280 developing fruits aborted from *C. pentandra* trees were collected from the soil and inspected looking for insect larvae. From this sample, 53% of flower buds and 14% of developing fruits contained curculionid beetle larvae in different developmental stages. The beetle species *Lonchophorus fusiformis* (Subfamily Anthonominae) was only found in closed flower buds and *L. santarosae* was found in developing fruits (FERNÁNDEZ et al. 2008).

### Pollen germination and pollination experiments

Pollen germination experiments demonstrated that pollen grains were able to continue germinating, although in low quantities, until 60 hours after they were removed from flowers (Fig. 3). It was possible to observe that during the first 14 hours after pollen grains were removed from flowers, the germination rate was relatively high with values around 60% to 70%. However, when pollen removal time increased, we observed a decrease in the germination rate that reached values near zero after 72 hours (Fig. 3).

The abundance of pollen grains in stigma and pollen tubes at the end of the style in flowers from pollination experiments (self, cross and natural pollinations), is shown in Figure 4. Flowers given natural and cross pollination treatments showed high and similar mean numbers of pollen grains germinated in stigma area. Also, when we analysed the number of pollen tubes at the end of the style, we found that their abundance in flowers from self-pollination, cross pollination and natural pollination treatments was also very similar in terms of mean and standard deviation (Fig. 4).

Finally, fruit set obtained for pollination treatments showed that 25% of flowers on which we performed cross-pollination set fruit. Contrarily, flowers which un-

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derwent self-pollination did not produce fruit. Fruit set of 10% was obtained for flowers with natural pollination treatment.

### Floral visitors

The following bat species were captured in mist nest carrying *C. pentandra* pollen: *Glossophaga soricina* (n = 12, Fig. 4), *Uroderma bilobatum* (n = 9), *Artibeus phaeotis* (n = 2) and *Carolia perspicillata* (n = 2). The great number of bats flying around flowering trees which is commonly observed for trees of this species located in the dry forests of Guanacaste, NW Costa Rica was not reflected in our study.

## Discussion

We observed that phenological cycles of *Ceiba pentandra* are irregular and showed great variability in flowering and fruiting intensity among individuals and reproductive episodes, in accordance with previous descriptions for this species in the literature (BAKER 1983, FRANKIE et al. 1974, MURAWSKI & HAMRICK 1992, NEWSTROM et al. 1994). Based on our data, we found that some individuals reproduced every year, while many others showed a variable duration between each flowering and fruiting episode. Most *C. pentandra* trees lost and replaced their leaves every year. The production of flowers and fruit, however, were not as regular as leaf replacement. Although complete leaf abscission is necessary for the start of flowering activity, the loss of leaves does not necessarily predict the production of flowers on *C. pentandra* trees (ROJAS-SANDOVAL 2004).

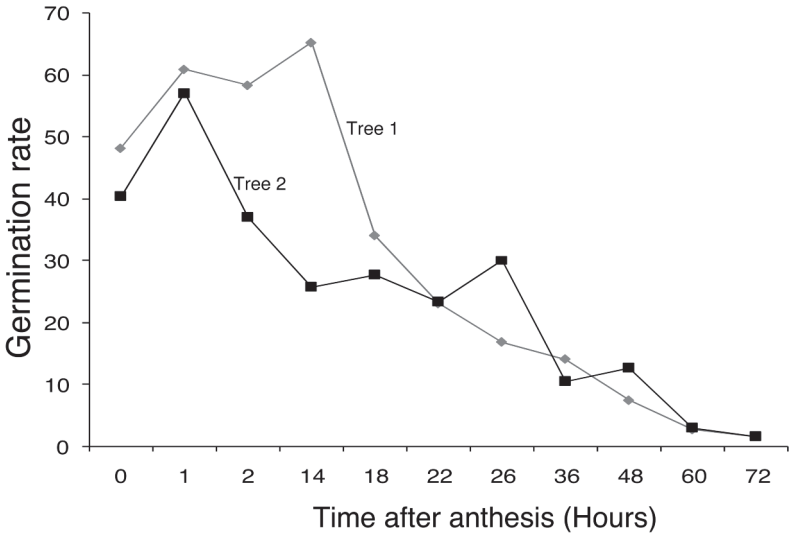


**Fig. 3**: Pollen germination rate for *Ceiba pentandra*. Data were collected from two trees and germination rates were obtained at different times after flower anthesis.

In this species, leaf abscission date was relatively invariable from one year to the next, while flower initiation date was more variable. The onset of leaf abscission varied within a week, while the onset of flowering varied within three weeks across the three years of study. Therefore, periods between leaf loss and flowering activity are variable, and might be determined by the presence of complementary abiotic signs that trigger the maturation of flower meristems. We found that timing and intensity of flowering activity were associated in one of the three years of study. In 2003, when there was

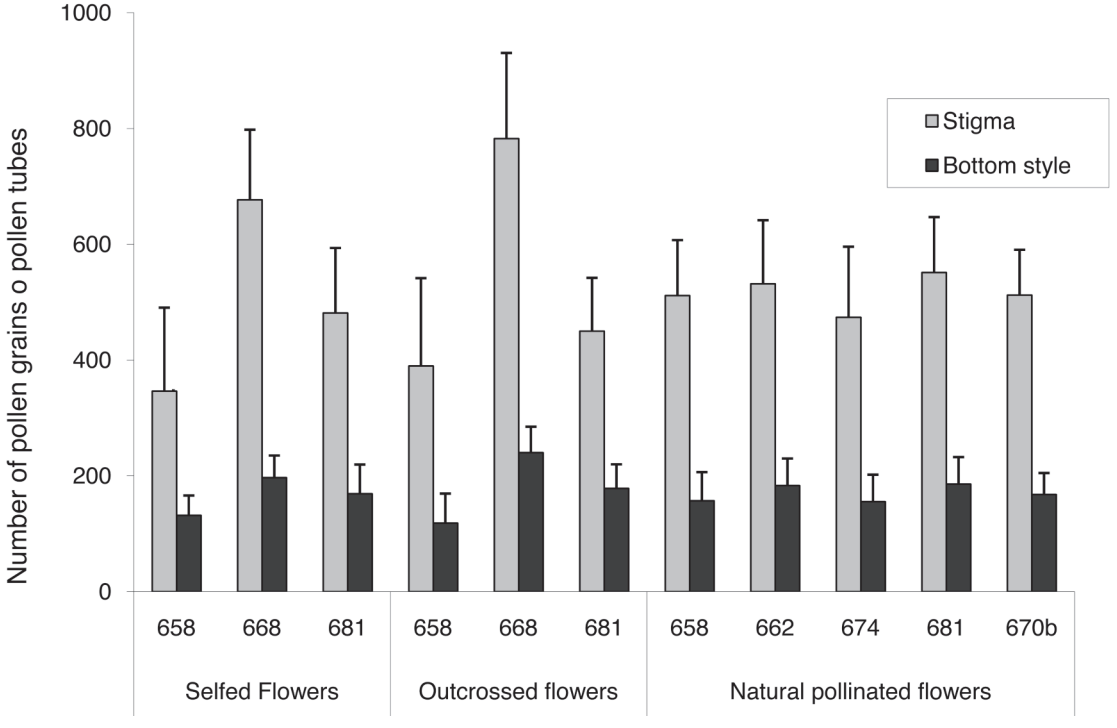


**Fig. 4**: Number of pollen grains or pollen tubes in flowers from different *Ceiba pentandra* trees. Each column represents the mean of pollen grains (stigma) or number of pollen tubes (bottom style) in 20 flowers from different pollination treatments (self-pollination, outcrossing or natural pollination). Horizontal axis numbers indicate the identity of trees where pollination treatments were performed.

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**Fig. 5**: *Glossophaga soricina* bat captured in a mist net located near a *Ceiba pentandra* tree in Palmar Norte, SE of Costa Rica. The presence of abundant yellow pollen typical of this tree species is observed in face, chest and wing.

an advance in the date of the onset of flowering, there were also high levels of flower production.

Although the timing of events is highly regular and synchronised at the population level for C. *pentandra*, individual phenology is a very irregular process. Although trees were subject to very similar environmental conditions of humidity and light, their phenological behaviours were often completely different (Figure 1). These observations suggest that the phenological behaviour of C. *pentandra* may be regulated by endogenous or internal cycles, which vary among individuals within the same population of trees. Years with exceptionally high flowering activity did not translate into years with great reproductive output, because there was massive abortion of flower buds and developing fruit.

Our results showed that a high proportion of aborted flower buds and developing fruits were attacked by different species of curculionid beetles. Indeed, high rates of abortion caused by insects may not only help us to explain the events in which trees lose all their flower buds and developing fruit within one year, but could also be a selective pressure for the evolution of massive and discontinuous flowering over the years. We propose that the production of a surplus of flower buds and developing fruits in C. *pentandra* can be an adaptation to satiate predators of reproductive organs (JANZEN 1971).

The similar number of pollen tubes in flowers with self, cross and natural-pollination treatments suggests that for this species, incompatibility barriers are not present at stigma-style levels. As a result, there is no mechanism at these levels that can inhibit germination of self-pollen and growth of pollen tubes, as is suggested by GRIBEL et al. (1999). However, our results show that fruit set for flowers with self-pollination treatment is very low or absent, whereas fruit set for flowers with cross-pollination treatment is about 25%. These results are similar to results obtained by GRIBEL et al. (1999) for C. *pentandra* trees in Central Amazonia. This species seems to have a self pollen rejection mechanism at ovary level or a high mortality of self-pollinated embryos, and the two possibilities are difficult to separate. On the other hand, our result suggests that fruit production in C. *pentandra is* partially limited for the arrival of exogenous pollen, due to differences in fruit set for cross-pollinated flowers and naturally pollinated flowers (10%), and the copious quantity of pollen grains observed in flowers with natural pollination treatment.

Our study shows that pollen for this species is viable for several nights and this issue may promote outcrossing by pollen carry-over, since pollen would remain viable in bats' fur for future visits to other trees. However, it is necessary to study the effect of grooming behaviour on the possibility that pollen present in bats' fur during one night be available for floral fecundations on subsequent nights.

In the dry forest of Guanacaste (Costa Rica), bat species that have been observed visiting C. *pentandra* trees are: *Glossophaga soricina*, *Glossophaga leachii*, *Phyllostomus discolor*, *Phyllostomus hastatus*, *Artibeus jamaicensis*, *Artibeus phaeotis*, *Artibeus intermedius* and *Sturnira lilium* (HEITHAUS et al. 1975, LOBO et al. 2005). Likewise, the list of bat species that visit C. *pentandra* flowers in the wet forest of SE Costa Rica include nectarivorous, frugivorous and generalist species that benefit from this resource during the dry season. However, in the Osa Peninsula, we did not observe a great number of bats of large size (i. e., *P. discolor*) flying around trees and visiting flowers almost all night long, as is common to observe for trees of this species located in the dry forests of Guanacaste (LOBO et al. 2005). These results may be explained by differences in relative humidity for the dry season between Guanacaste and the Osa Peninsula, that convert a flowering episode of C. *pentandra* into a more attractive event in the dry forest than in the wet forest. In addition to bat species, we observed kinkajous (*Potus flavus*) visiting and feeding pollen and nectar in C. *pentrandra* trees located at the Corcovado National Park and near Palmar Norte Town (ROJAS-SANDOVAL 2004).

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