Pollination Biology of Calotropis procera subsp. hamiltonii (Asclepiadaceae)

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With 4 Figures

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Summary


The main pollinators of Calotropis procera (Ait.) AIT. f. subsp. hamiltonii (Wight) ALI in the area of Karachi are males and females of Xylocopa pubescens SPIN. and females of X. fenestrata (F.) (Anthophoridae). Workers of Apis florea F. (Apidae) are minor pollinators. The activity of the pollinators (measured as the number of pollinated flowers) was very different in the three populations investigated. The opening of stigmatic chambers by insect legs is regarded as a presupposition for the insertion of a pollinium into the chamber. Into stigmatic chambers whose proper corpusculum is present, a pollinium is more easily inserted than in those chambers whose pollinarium was removed. Pollination of a flower with one pollinium is sufficient for the development of a follicle. The pollen-ovule ratio is low (6.14).

Zusammenfassung


1. Introduction


The present paper is concerned with the study of several aspects of pollination biology of Calotropis procera (Ait.) Ait. f. subsp. hamiltonii (Wight) Ali. This taxon, widely distributed in Pakistan, is very common along the roadsides and in other disturbed habitats all over Karachi and its vicinity. It is an erect shrub or a small tree up to 3 m. tall, much branched from the base. Young stems and leaves are covered with white cottony tomentum. Flowers are in terminal or axillary umbelliferous cymes. The plants flower all the year round.

2. Material and Methods

All the observations are based on the study of fresh material.

Determination of number of pollen grains per pollinium:

The pollinia were soaked in water overnight. Due to hydration, the pollen masses become loose and by teasing the pollinium wall with dissecting needles pollen grains easily get separated from each other and may be counted easily (KHATOON & ALI 1983).
Determination of number of ovules:
The number of ovules was determined by counting the ovules in the ovaries (under a dissecting microscope) of the same flowers as used for the pollen grain count.

Level of pollinator activity in natural populations:
Random samples of flowers were collected from different natural populations, all located in the area of Karachi: (i) The vicinity of Karachi University Campus (ii) Darsano Chino and (iii) Mangopir. The flowers were preserved in 50% ethyl alcohol. Later these flowers were studied (under a dissecting microscope) in order to determine the number of pollinaria removed and the number of pollinia inserted into the stigmatic chambers. Flowers were also counted for insertions into chambers flanked by intact versus removed pollinaria.

Insects:
The insects that visited the flowers were collected and examined. The study was made in Karachi from the above mentioned localities from 15th April to 20th October 1985; it was limited to the periods when weather conditions were favourable for the foraging activities of the visitors. All insects were taken between 8.0 a.m. and 7.0 p.m.

The identification of insects was made as per direction of Dr. Laurence A. MOUND (British Museum, Natural History), London.

3. Floral Morphology

Five green sepals surround the five petals with purple tips. The corona is made up of five fleshy, purple, laterally compressed lobes radiating from the gynostegium (Fig. 1A). Each lobe consists of a recurved vesicle at the base and a bifid apex with a cleft outside (Fig. 1B). The gynoecium consists of two free carpels which are united at the apex of the style. The “stigma” is broad, pentagonal and the true stigmatic surfaces are enclosed below the stigmatic lobes within the stigmatic chambers between adjacent anthers.

The androecium consists of five stamens whose filaments are fused to form the column. The pads are part of the column and fit between the corona lobes. Each anther is bilocular and with a hyaline outhgrowth of the connective that covers the stigmatic disc at the periphery (Fig. 1B). Each anther sac contains a pollinium. There are five pollinaria, each consisting of paired pollinia from adjacent anthers jointed by translator arms to a corpusculum located just above the slit or opening of the stigmatic chamber (Fig. 1. B, C). The stigmatic chambers are beaklike, due to raised anther flaps (wings). Anther flaps are hard, straight and enclose the stigmatic chamber tightly.

Each pollinium is a flat wing-like body, narrow at its base close to the translator and wide at the apex. It has no external appendages and no pellucid margin. The pollinium is pendulous in transport.

The germination locus is situated in the broadest portion of the pollinium and it is not surrounded by any external appendages (Fig. 1C and 4c). When the pollinium is inserted in the stigmatic chamber the germination locus coincides with the receptive surface of the stigma i. e. the exolateral
Fig. 1. A. Flower of *Calotropis procera* subsp. *hamiltonii*. – B. Side view of the gynostegium and two corona lobes. The arrow indicates the portion below the anther flaps (wings) by pressing of which the stigmatic chamber is opened. Dotted lines indicate the position of pollinia within the anther sacs. – C. Pollinarium. – ac apex of corona lobe, af anther flap, c corpusculum, cl corona lobe, ex exolateral surface, hc hayline outgrowth of connective, pa pad, pe petal, po pollinium, s sepal, sc stigmatic chamber, sd stigmatic disc, ta translator arm, v vesicle.

side of the pendulous pollinium is located towards the receptive site. In *C. procera* subsp. *hamiltonii*, generally the whole pollinium is inserted in the stigmatic chamber; rarely it extends partly or entirely outside the flaps of the stigmatic chamber.

### 4. Pollen – Ovule Ratio

The average number of pollen grains per pollinium is $350 \pm 2.75$ (N=30) and the average number of ovules per ovary is $285 \pm 22.4$ (N=40). Thus, because there are 10 pollinia and two ovaries per flower, the pollen-ovule ratio is 6.14.

### 5. Level of Pollinator Activity

The Darsano Chino population has the highest level of pollinator activity as compared to the populations of Mangopir and Karachi University Campus. This can be concluded from the percentage of pollinated flowers (Tab. 1).
Table 1. Percentage of pollinated flowers

<table>
<thead>
<tr>
<th>Localities</th>
<th>No. of flowers examined</th>
<th>Pollinated flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karachi University Campus</td>
<td>225</td>
<td>4.44%</td>
</tr>
<tr>
<td>Mangopir</td>
<td>45</td>
<td>4.44%</td>
</tr>
<tr>
<td>Darsano Chino</td>
<td>50</td>
<td>48.0%</td>
</tr>
</tbody>
</table>

The 320 flowers examined all together have 1600 stigmatic chambers. The result of their investigation is summarized in Tab. 2. In the Darsano Chino population in c. 16% of the stigmatic chambers inserted pollinia were present, in the two other populations only in 0.8% of the chambers. The pollinaria appertaining to the investigated stigmatic chambers were removed at ca. 45% in Darsano Chino population and only at 3% and 7% respectively in the other two.

Table 2. Percentage of removed pollinaria and inserted pollinia

<table>
<thead>
<tr>
<th>Localities</th>
<th>No. of stigmatic chambers</th>
<th>Intact pollinarium present</th>
<th>Pollinarium removed</th>
<th>Inserted pollinium present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karachi University Campus</td>
<td>1125</td>
<td>96.9%</td>
<td>3.0%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Mangopir</td>
<td>225</td>
<td>92.8%</td>
<td>7.1%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Darsano Chino</td>
<td>250</td>
<td>54.4%</td>
<td>45.6%</td>
<td>16.4%</td>
</tr>
<tr>
<td>Total</td>
<td>1600</td>
<td>89.7%</td>
<td>10.2%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

If pollinaria were removed, there were usually 2–3 per flower in the Darsano Chino population and nearly one in the Campus and Mangopir populations.

Out of the stigmatic chambers with inserted pollinia, 33 were accompanied by their intact pollinarium whereas in 19 cases the pollinarium was removed.

6. Pollinators

Insects bearing pollinaria were classified as pollinators. The pollinators include the following Hymenoptera:

- Anthophoridae, Xylocopa pubescens SPIN., males and females (Fig. 2a, b);
- Xylocopa fenestrata (F.), females (Fig. 2c);
- Apidae, Apis florea F., females (Fig. 2d, e).
Both the species of Xylocopa together constitute 82.75% of the total number of all the pollinaria bearing visitors while Apis florea (F.) represents 17.25%.

Nectar appears to be the main floral reward.

6.1. Xylocopa species

The two Xylocopa species, X. pubescens SPIN. and X. fenestrata (F.) exhibited a diurnal foraging pattern. The first visits to the plant start at about 7.30 a.m. and continue to about 7.0 p.m. (particularly in May when flowering was intensive and insects were abundant). Xylocopa species gradually increase in number from about 9.0 a.m., reaching peak frequency between 10.0-11.0 a.m., fluctuating at this level from 12.0-2.0 p.m. and again reaching the peak frequency between 3.0-5.0 p.m. and declining slowly thereafter.

The Xylocopa species land on the flower particularly on the broad stigmatic disc. Front legs usually grasp the corona lobe from the base while the middle and hind legs often rest over the anther wings and stigmatic chambers. Particularly tarsal segments of legs frequently come in contact with the corpuscula during foraging. As a result, the corpusculum gets attached to a leg hair and removal of pollinarium occurs. When the insect visits another flower the pollinium can be lodged in the stigmatic chamber.
The emphasis seems to be on the nectar gathering. The insect inserts its proboscis in the opening at the apex of the corona lobe to suck the nectar. During nectar sucking, the insect tries to hold its grip on the staminal column or applies force to press the pads so that the nectar may rise up in the corona lobe (Fig. 3). Probably this grip also helps in generating the force required for penetrating his proboscis between the coronar lips strongly pressed together.

Fig. 3. Xylocopa pubescens ♀, inserting its proboscis in the opening at the apex of the corona lobe to suck the nectar.

Fig. 4. A, B. Pollinaria attached to tarsal segments. – A Xylocopa pubescens ♀, B Apis florea ♀.

Fig. 4 C. Dissected stigmatic chamber of Calotropis, showing an inserted pollinium with the germination locus at the exolateral surface and pollen tubes penetrating into a style.
Xylocopa usually exploits one nectary of a flower, but sometimes also two or three. The bee prefer to fly from one plant to another plant and also to another inflorescence of the same plant; very rarely Xylocopa changes to other flowers of the same inflorescence.

The number and location of the pollinaria and the corpuscula position (either directly attached to the insect body or indirectly, i.e. attached to another translator) on different parts of the insects are examined (Tab. 3). X. pubescens (♂ & ♀) carried 17.9% and 14.3% of the pollinaria (out of the total pollinaria carried by all insects), X. fenestrata (♀) carried 42.5% of the pollinaria. X. pubescens (♂) carried not a single indirectly attached corpusculum (N=30). 91.6% directly and 8.3% indirectly attached corpuscula were carried by X. pubescens (♀), 94.3% and 5.6% respectively by X. fenestrata (♀). The largest number of pollinaria are located on tarsal segments (Fig. 4A) and fewer on tibiae and claws while none on the mouth parts (Table 3).

Because of its abundance on the flowers of C. procera subsp. hamiltonii (more than 80% of all pollinator visits counted) and ability to remove and insert pollinaria, the Xylocopa species should be the main pollinator of this plant.

Table 3. Location and number of pollinaria attached to pollinators. Frequency and location of directly (d) and indirectly (i) attached corpuscula

<table>
<thead>
<tr>
<th>Insect</th>
<th>No. of specimens examined</th>
<th>Mouth Parts</th>
<th>Claws</th>
<th>Tibiae</th>
<th>Tarsi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d</td>
<td>i</td>
<td>d</td>
<td>i</td>
</tr>
<tr>
<td>Xylocopa pubescens ♂</td>
<td>5</td>
<td>30</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Xylocopa pubescens ♀</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Xylocopa fenestrata ♀</td>
<td>28</td>
<td>64</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Apis florea ♀</td>
<td>10</td>
<td>32</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>57</td>
<td>147</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.2. Apis florea

Apis florea (F.) workers visit the flowers the whole day and land on the flower particularly on the stigmatic disc. The insect inserts its proboscis in the opening at the apex of corona lobe to suck the nectar; sometimes the insect tries to suck the nectar from the recurved vesicle also, but without any success. During foraging, the body of the insect rests on the stigmatic disc while its legs particularly the middle and hind legs rest over the stigmatic chambers. Tibial and particularly tarsal segments of legs of the insect frequently come in contact with the stigmatic chambers and corpuscula.
Apis florea carried 25% of the pollinaria counted for all insects. 95.2% were directly attached and 4.7% indirectly. Usually the pollinaria are attached to the tarsi (Table 3, Fig. 4B).

7. Non-pollinating insects

Abundant visitors of flowers were members of Hymenoptera (Vespidae sp.; Amegilla comberi, Anthophoridae) and Homoptera (Spilostethus pandurus (SCOPOLI), Lygaeidae; Fig. 2h).

Amegilla comberi (CKLL.) individuals have two types of foraging behaviour. Firstly it does not land on the flower but remains in the air by vigorous wing movement and sucks up the nectar from the opening at the apex of corona lobe through its long proboscis. Secondly, it lands on the flower between the two petals, front and middle legs grip the petals from above and hind legs from below, inserting its long proboscis in the opening at the apex of a corona lobe to suck the nectar. A. comberi was a rare visitor.

Vespidae species (males und females, Fig. 2f, g) have been observed on the plant all the year round and were abundant. They are also found on the plant during the dry (winter) season. The insect lands on a petal of the flower and sucks the nectar from the base of the pads. They are usually also observed on both the surfaces of the leaves. The plant acts as the site for their copulation also.

Rare visitors of the flowers were some Diptera (small flies) and Lepidoptera (small butterflies). All those insects may be regarded as opportunists that merely exhaust the floral rewards without providing pollination.

8. Pollinaria Removal and Pollinia Insertion Mechanisms

Examination of the pollinaria removal mechanism in C. procera subsp. hamiltonii indicates that during foraging the insects press the pads. The pressing and repressing of the pads involves the movement of legs over stigmatic chambers. In this process tarsal and tibial segments of the insect's leg frequently come in contact with the corpuscula. Thus a corpusculum gets attached to a leg hair and along with the leg the corpusculum with the attached pollinaria is pulled upward freeing the pollinia from their anther sacs.

As stated earlier, the stigmatic chamber is beak like and tightly closed by thick and hard anther flaps, so that the opening of the stigmatic chamber must be the first step in the process of pollinium insertion. Observations revealed that during the pressing of the pads by the insect, the portion above the pads and below the anther flaps of the stigmatic chamber (arrow in Fig. 1B) is also pressed, as a result anther flaps get apart (are separated) and the stigmatic chamber opens. The previously removed pollinaria becomes posi-
tioned on the hair of the insect's leg through the curvature of the translator arm so that the exolateral surface of the pollinium with the germination locus extends away from the insect's leg. The pollinium (not the translator arm) enters into the stigmatic chamber and the translator arm breaks out at the bend below the corpusculum as the insect lifts its leg, leaving the pollinium within the stigmatic chamber.

Two factors seem to be responsible for the breakage of the translator arm at the knee bend:

(i) Presence of an intact corpusculum: when the translator arm of the inserting pollinium comes in contact with the intact corpusculum, the groove of the intact corpusculum acts as a point of resistance and the break at the knee bend occurs, as the insect pulls its leg (Wyatt 1976).

(ii) Closing of the stigmatic chamber: Another plausible reason for the successful pollinium insertion into the stigmatic chamber in the absence of the intact pollinarium may also be possible. As an insect stops pressing the pads, anther flaps come back to their original position, the stigmatic chamber is closed and grasps the inserted pollinium. When the insect pulls its leg, the break occurs at the knee bend.

9. Efficiency of Pollination

Formerly it was believed that insertion of two pollinia into adjacent stigmatic chambers of a flower is necessary to affect fertilization in Asclepias (Moore 1946, 1947). Sparrow & Pearson 1948 stated that the two pollinia must be located in the stigmatic chambers in such a way that the pollen tubes from one pollinium will be directed towards one carpel and those from the other pollinium towards the second carpel given that the two adjacent chambers belong to one carpel and the other three to the second carpel. However, in C. procera subsp. hamiltonii, field and laboratory observations indicate that the insertion of a single pollinium into a stigmatic chamber of a flower is sufficient to affect fertilization (Fig. 4c). Whether or not double insertion (opposite or adjacent) increases the probability of fruit set in one or both carpels remains to be tested. It seems that commonly one pollinium fertilizes a single ovary but very rarely, as observed in one case, both the ovaries may be fertilized by a single pollinium. This latter fact is in accordance with the presence of a compitum (possibility for pollen tubes to reach both styles from one stigmatic chamber).

10. Discussion

Descriptions of pollination ecology of Calotropis are mainly those of Jaeger 1971 (Xylocopa sp. in Senegal), Schremmer 1972: 273–278, 5 figures (Xylocopa leucothorax main pollinator in Egypt) and Eiskowitz (X. pubescens and X. sulcatipes only pollinators in Isreal) for C. procera and for C. gigantea by van der Puij 1954: 554–556,

From the point of view of flower ecology the most apparent differences between C. procera and C. gigantea are the large flowers of the latter (diameter ca. 5 cm instead of 1.5–2 cm in C. procera) and another accessibility of the nectaries (entrance not at the apex but by small openings at the base of the corona lobes in the involutions under the tips of the nectar containing spurs, see WANNTORP).

VAN DER PIJL 1954 has given a full survey of flowers apparently adapted for pollination by Xylocopas (flowerclass Xylocopa blossoms) and mentions (p. 559) nine characteristics of them, mainly flowers spacious, solid, nectar hidden and mostly accessible only by force, strong walled nectar room separated from an ante-room, nototribic pollen application, nectar not abundant. SCHREMMER 1960 and 1972 has contributed much to the understanding of Xylocopa blossoms. VAN DER PIJL 1954: 554 and SCHREMMER 1972: 277 wonder that flowers so different from usual Xylocopa blossoms like the Calotropis flowers are nearly exclusively visited by Xylocopa. But if one regards (according to SCHICK & REMUS 1984) the five sectors of the flowers as meranthia, the deviation is not so spectacular; then – the nototribic condition excepted – all essential characteristics stated by VAN DER PIJL are realized.

The two subspecies of Calotropis procera differ only in fruit characters (ALI 1980). In Calotropis procera subsp. procera the fruit is globose 10.8–14.5 cm long and 9.7–11.7 cm broad. The tip is invaginated at maturity and the width of the air space of the loculus is greater than the central seedbearing area and the placenta, whereas in C. procera subsp. hamiltonii the fruit is shaped like the neckless inverted body of a duck, 6.5–11.7 cm long and 3.0–5.1 cm broad. The tip is not invaginated at maturity and the breadth of the air space is less than the breadth of the central seedbearing region and the placenta.

Calotropis procera subsp. procera is present in tropical to dry sandy parts of Africa, extending the Mediterranean belt, Jordan, Arabia, Palestine, Abu Dhabi and introduced into the West Indies and tropical South and Central America. C. p. subsp. hamiltonii is found within Pakistan, India, Afghanistan, Iran (?), Iraq (?) and Oman (ALI 1980, 1983).

The observations on pollination of C. p. subsp. procera reported by SCHREMMER 1972 are essentially in accordance with ours; in Egypt X. leucothorax (exclusively males) were the main pollinator and a Scoliid wasp a facultative one. In X. pubescens we have found males and females, in X. fenestrata only females acting as pollinators. X. pubescens pollinates Calotropis flowers in Israel also (EISIKOWITCH 1986).

The percentage of the removal of pollinaria is comparatively high in the Darsano Chino population (45.6%) as compared to the Karachi.
University Campus and Mangopir populations (3.0% and 7.0% respectively (Tab. 2). Lynch 1977 pointed out that as the number of pollinaria removal increases, it should indicate increasingly higher levels of pollinator activity. Thus, the Darsano Chino population has a high level of pollinator activity. The differences in the levels of removal and insertion of pollinia may be due to the availability of the pollinators, colony size, nearest colony and nearest neighbour plant distances, inflorescence size etc. and due to some other parameters (Wyatt 1976).

The pollinium insertion mechanism in C. procera subsp. hamiltonii is similar to that described by Wyatt 1976 and Beare & Perkins 1982 for Asclepias tuberosa except for the mechanism of opening and closing of the stigmatic chamber.

Of the 1600 stigmatic chambers (from all three populations) for which the presence or absence of pollinia was recorded, 10.25% of the pollinaria had been removed and only 3.25% of these stigmatic chambers contained an inserted pollinium (Tab. 2). There is a significant difference (P<0.01) between the insertions flanked by intact pollinia (N=33) and those not flanked by intact pollinia (N=19). Thus our observations provide data in support of Wyatt’s 1978 hypothesis that successful pollinia insertions are primarily into the stigmatic chambers flanked by intact pollinia (compare the experiments by Wyatt 1978 and Beare & Perkins 1982 in Asclepias).

The pollen-ovule ratio (P/O) is defined as the ratio of pollen grains produced per ovule (Cruden 1976). The P/O in Asclepiadaceae, studied by Cruden 1977 varied from 3.8—10.8. While in C. procera subsp. hamiltonii, the P/O is 6.14. Thus, our data support Cruden’s 1977 observations that Asclepiadaceae have low P/Os as compared to other xenogamous species.

11. Acknowledgements

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12. References


