SPECIES

Insights on the flower behaviour and trap mechanisms of *Aristolochia ringens*, gaping Dutchman's pipe

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ABSTRACT

Pollination biology and flower behaviour were studied in *Aristolochia ringens* Vahl (Aristolochiaceae) ex-situ at St. Joseph's University Campus, Bengaluru. The flower is a two-day bloom and is strongly protogynous. On day one the stigma is receptive and the odour of the flower is strikingly high. On day two the stigma receptivity is lost and dehiscence of anther takes place along with several other changes in the flower. Five flower visitors were seen in close contact with the flower. The common housefly, bluebottle fly and flesh fly were attracted to the colour or odour of the perianth with repeated abdominal strikes onto the perianth flap while in flight. Whereas, *Limnophora* sp. and Psyllid fly were found inside the perianth chamber. The morphological and anatomical changes of the flower were studied with reference to plant-pollinator interactions.

Keywords: Flower traps, *Aristolochia ringens*, Sapromyiophily, Gaping Dutchman's pipe trap.

1. INTRODUCTION

The genus *Aristolochia* (Aristolochiaceae) has a specialised pollination system with flowers representing insect traps (Aliscionia et al., 2017) and is predominantly sapromyiophilous in nature. Here the transport of pollen to the stigmatic surface is taken care of by flies usually after the entrapment. Flesh-coloured flowers, decaying obnoxious odour and excessive downward-pointing hair near the trap distinguish these flowers. In all *Aristolochia* species reported so far, flies of different families, including Anthomyiidae, Chloropidae, Milichiidae, Phoridae, Sarcophagidae and Syrphidae, have been recorded as pollinators (Cammerloher, 1923; Petch, 1924; Brues, 1928; Lindner, 1928; Brantjes, 1980; Costa and Hime, 1983; Wolda and Sabrosky, 1986; Hall and Brown, 1993). The flowers are strongly protogynous they open on the first day when the stigma remains receptive. While, on the second day the anthers dehisce and shed pollen (Sakai, 2002).

Most of the *Aristolochia* spp. deceive their pollinators and trap them; however, *A. inflata* and *A. maxima* offer breeding sites for the pollinators (Sakai, 2002). The percentage of open pollination is lesser than the manually pollinated flowers, suggesting that the mode of pollination is an important factor for fruit set in the *ex-situ* grown populations of *Aristolochia tagala* (Murugan et al., 2006). Several species of *Aristolochia* are medicinally important. Roots, leaves and whole plant of *A. ringens* are used to treat diverse diseases like typhoid, skin diseases, sores, ulcers, guinea worm, fever, asthma, hemorrhoids, diabetes, an antidote to snakebites and root extract also shows high anticancer properties (Van-Wyk and Wink, 2004; Olabanji et al., 2008; Sonibare and Gbile, 2008; Aigbe and Adeyemi, 2011; Soladoye et al., 2011; Akindele et al., 2014). To our knowledge, there are no studies on the flower reproductive biology and trap mechanisms of *A. ringens*. This communication reports the results of our studies on the flower trap mechanism and flower biology of *A. ringens* grown under ex situ conditions in Bengaluru.

2. MATERIALS AND METHODS

Saplings of *Aristolochia ringens* had been introduced at St. Joseph's University Campus (SJUC) at Bengaluru (lat. 12°57' N, long. 77°35' E; altitude 900 m asl). They had established well and were flowering regularly. Each vine produces a large number of flowers (>50).

Direct observation of the plant

The study plant was observed for five hours every day, five days a week for two months (Jan – Feb 2020) to understand the floral biology of the plant. All the stages of the flowers, flower visitors and parts of the flowers, were photographed using Sony Cyber-shot DSC-H300.

Flower biology

The flower is very uniquely evolved to trap insects especially flies to bring about an important life process, pollination. Flowers were solitary in axils and pendulous; pedicel (7.5-17cm), at the flower tip pedicel had a characteristic six-ridged appearance. The perianth bulges to form an obovoid utricle which was speckled with purple markings, and perpendicular to the utricle is the limb. The limb was bilabiate with an upper spathulate lid (Figure 1, yellow arrow) and a perpendicular long flapped tube (Figure 1, white arrow).

The Flower phases

The flower bud develops in a very sophisticated manner. Flower bud to a completely mature bloom has six phases. *Phase 1*- the bud was completely green and no perianth markings were seen, the perianth limb tube completely incurved and touched the base of the utricle and the tube was 1.2 ± 0.5cm (Figure 5). *Phase 2*- the bud had faint perianth markings and the limb tube incurved but seldom touched the base of the utricle. The utricle had more green than purple markings; the tube was 2.26 ± 0.15cm (Figure 6). *Phase 3*- the marking on the utricle deepens, but the markings at the tip of the utricle remain green and the length of the tube was 4.6 ± 0.16cm (Figure 7). *Phase 4*- the perianth markings further deepen, the perianth tube and limb were more purple than green and the tube comes perpendicular to the utricle. It was measured to be 7.46 ± 0.15cm (Figure 8). *Phase 5*- the flower was completely matured in this phase, the utricle, limb tube and lid together had more purple than green, the limb tube was completely perpendicular and the limb flap/lid was 60 degrees to the utricle. The measured limb tube length was 12.63 ± 0.16cm. It emanates a strong odour in this phase (Figure 9). *Phase 6* – those flowers which were not visited by pollinators in the last phase pass this phase. Very few flowers in the study area passed this phase. The limb tube length was 15.6 ± 0.5cm

Utricle window

At the point of attachment of the utricle and the pedicel, there was a region that was devoid of markings or had faint markings. It was more hyaline than green. This area mimics the window structure. The hyaline nature of this region could be due to the translucent cells that make up this region (Figure 23, black arrow). It is delimited from the other cells of the utricle by ten to twelve layers of purple cells; this encirclement of purple cells borders the window and other utricle areas. The gynostemium centres the window area (Figure 14) and the pollinator is deceived by this relatively lit area. It repeatedly comes to the window hoping for an escape, thereby contacting the reproductive organs. On the second day, it gets dusted with the pollen grains and escapes from the entrance it came in, as the trichomes wither away and unseal the trap.

Plate - 1

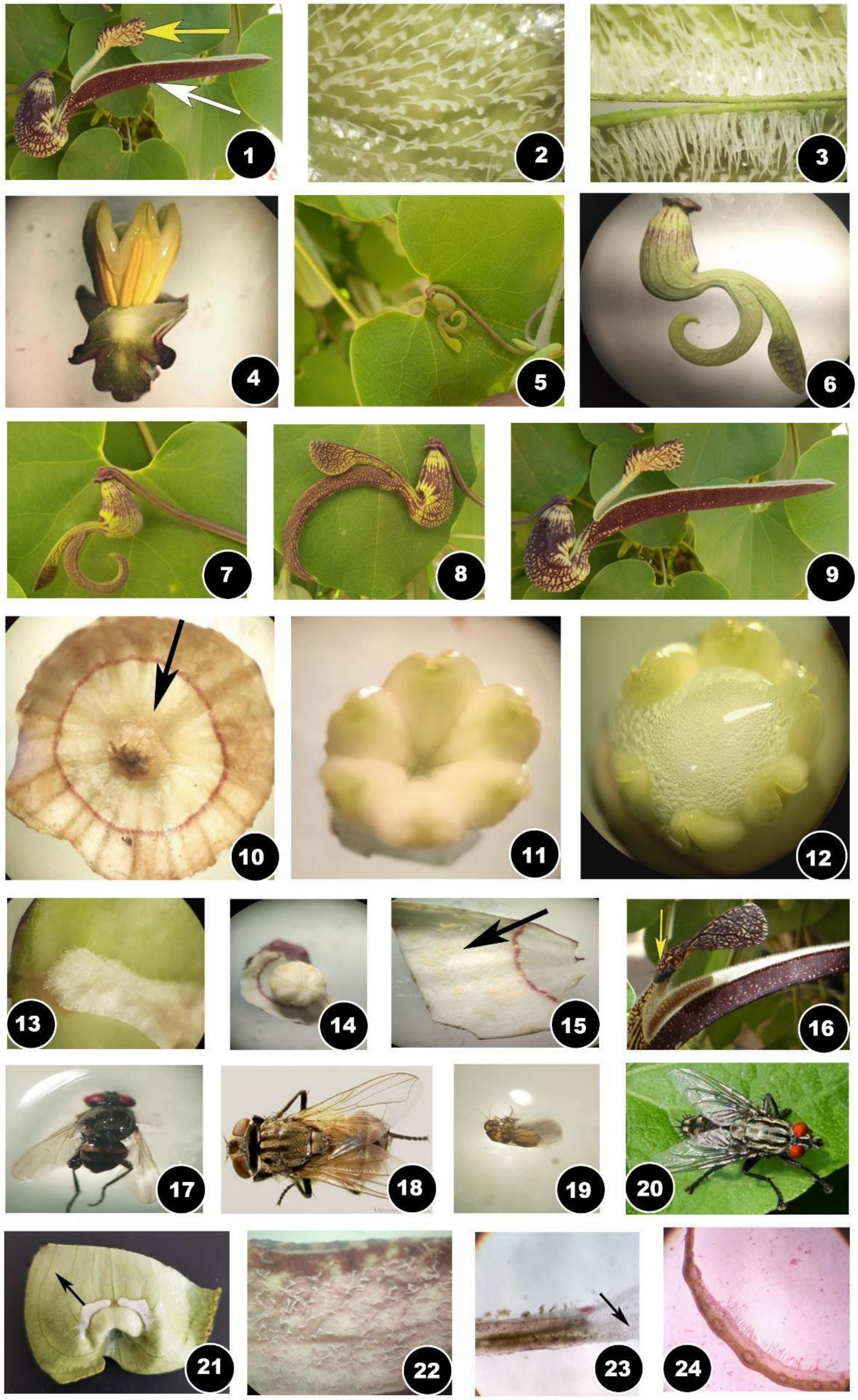

Plate 1 Figure 1: Flower showing lid (yellow arrow) and tube (white arrow); Figure 2: Downward pointing hairs on the inner sides of the tube; Figure 3: Straight hair near the margins of the flap; Figure 4: First day gynostegium; Figure 5: Flower bud in phase 1; Figure 6: Flower bud in phase 2; Figure7: Flower bud in phase 3; Figure 8: Flower bud in phase 4; Figure 9: Flower bud in phase 5; Figure 10: Utricle window (arrow) bordered by a line; Figure 11: Gynostegium showing open stigmatic surface on the first day; Figure 12: Stigmatic surface showing positive for catalase test; Figure 13: Cottony mat surrounding the opening leading to the utricle cavity; Figure 14: Gynostegium with closed stigmatic surface on the second day; Figure 15: A strip of utricle showing anther dehiscence (second day); Figure 16: *Calliphora vomitoria* on the spathulate lid (yellow arrow); Figure 17: *Limnophora* sp.; Figure 18: *Musca domestica*; Figure 19: Sacrophagidae; Figure 20: A Psyllid bug; Figure 21: Utricle entry point surrounded by a cottony mat (black arrow); Figure 22: Inner side of the tube showing shrivelled trichomes; Figure 23: LS of the utricle showing translucent window cells; Figure 24: TS of the utricle showing numerous hairs on the inner side

The process

The flower behaviour was keenly observed concerning its pollination. When the flower was in phase 5, a thesis begins at 9:00 hrs ± 25 minutes IST, the perianth limb platform slowly opened up showcasing the multitudinous downward-pointing hairs (Figure 2) all through the blade surface except at the margins, where there are relatively long straight hairs (Figure 3) to ensure easy interlocking during closure. The utricle cavity emanated a putrid odor which was very intense through the utricle cavity orifice. Several flies were attracted to this obnoxious odor. Once the insect entered the orifice, the platform closes onto itself sealing any opening. If no insect visits the flower, it gradually starts sealing from 16:30 hours ± 30 minutes IST. On day one the anther is mature but does not dehisce, the stigmatic platform is fleshy, bloomed and secretes copious nectar, which increases with an increase in mechanical touch stimulus (personal observation). Stigma is receptive on the first day showing positive results for the catalase test (Figure 12).

On the second day, the stigma of the gynostemium opened on the first day now droops down on end-to-end margins and seals the stigmatic surface (Figure 14), nectar minimal or remnants of the day before. The anthers dehisced longitudinally, shedding copious pollen on the utricle wall; this wall had a lot of hair entrapping the pollen grain. The cottony white hair margining the utricle entrance becomes brown. Outside in the perianth limb platform, the hair wilts thereby the margins loosen out and produce a disjunctive space. The pollen loaded flower visitors escape from this opening.

3. RESULTS AND DISCUSSION

Our study species possess an elaborate trap-release mechanism that is in compliance with *A. grandiflora* (Cammerloher, 1923) *A. debilis* (Sugawara et al., 2016), where the downward pointing trichomes on the utricle and tube allow undemanding entry of the flower visitors but a challenging exit. It withers away when the flower changes its sex from female to male on the second day of its bloom, letting the flower visitor free with pollen load.

Our study species possess nectaries at the stigmatic out folds (Figure 4) of the gynostemium, but this is regarded as food for survival during captivity rather than a reward (Vogel, 1998). Also, there is no nectar secreted at the floral entrance as seen in many *Aristolochia* species. However, not all *Aristolochia* species produce food for their trapped visitors, flowers of *A. grandiflora* produce no food for the imprisoned flies and many dead flies were observed in the flowers (Petch, 1924). Many studies on the pollination systems of *Aristolochia* indicate that pollinator flies visit flowers because they are deceived by the obnoxious floral odor that mimics carrion, feces or fungi (Proctor et al., 1996). The foul stench at the orifice of the utricle of our plant directs the same.

4. CONCLUSIONS

Though the studied species is native to Argentina Northwest, Bolivia, Colombia, Costa Rica, Ecuador, El-Salvador, Panamá, Peru and Venezuela, we speculate that most of the species of *Aristolochia* have a trap mechanism of their own kind. Whenever these species are introduced into a new environment, information on whether or not they are pollinator deficient is crucial for the plant's survival. This, therefore, becomes crucial for documentation as this understanding can give us better information about the flower visitors and the pollination process, both being pivotal for plant survival and hence, conservation. This study becomes the first to document the trap mechanisms of *A. ringens* in urban landscapes.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for sample collection & identification.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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