

Pollination Biology of *Impatiens dalzellii* Hook. f. and Thomson (Balsaminaceae) Endemic to the Western Ghats, India

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ABSTRACT

The paper reports on the pollination biology of *Impatiens dalzellii*, a rare and endemic balsam of the Western Ghats. The plants flower during July to December with maximum bloom in October. The flowers last for 2-3 days. Anthesis occurs in night time (02.00-03.15 h) and dehisce one day before anthesis which confirms the protandrous nature of the flower. Pollen grains are spherical, tri-colporate with reticulate exine and 28.5µm in diameter. FCR test shows maximum pollen viability of 88% on the day of anthesis. The receptivity of stigma begins one day after anthesis and lasts until the late morning. Honeybees, hawk moths, butterflies and flies forage at the flower but honeybees serve a better pollinator. Pollen grains are deposited on the dorsal surface of the thorax during honeybee visit to the flowers and stigma rubs the pollen coated thorax and is pollinated when the bees visit the flowers of other plants. There is high level of xenogamy. Pollination efficiency under field condition is very poor which results in low fruit set but manual pollination enhanced the rate of fruit set up to 75%. Different pollination experiments confirmed that, the species is self-incompatible. None of the self-pollinated flowers sets fruits, but over 75% of the cross-pollinated flowers set fruits. Seed germination was also limited to 20%. The species is poorly distributed due to biotic and abiotic factors all of which either alone all in combination are responsible for the limited distribution of this species in the Western Ghats.

Key Words: Protandrous; Pollination Biology; Western Ghats; Conservation

INTRODUCTION

Balsams are included under the genus *Impatiens* L. belongs to the family Balsaminaceae, which is supposed to be one of the largest groups among flowering plants, comprising more than 1000 species globally (Grey-Wilson 1980, Clifton 2000, Yuan et al. 2004, Sreekala et al. 2008). They are mainly distributed in montane areas in the Old World tropics and subtropics (Grey-Wilson 1980, Yuan et al. 2004), but several species occur in temperate Eurasia and North America (Pieter et al. 2006). Native species are absent from the South America and Australia. In India, the genus is represented by 205 species (Vivekananthan et al. 1997, Vishwanathan et al. 2003, Bhasker 2006, Kulloli et al. 2008) and mainly distributed in three major centers of diversity i.e. Western Himalayas, hills of north eastern

states (Sikkim, Darjeeling districts of West Bengal and Arunachal Pradesh) and Western Ghats (Hills south of Tapti river in the north to Kanyakumari in the south, along the west coast of India). There are 95 species available in Peninsular India, Out of which 88 are endemic and confined to Western Ghats. Due to their restricted distribution, nearly 30 species of *Impatiens* are already in threatened category including 19 critically endangered with uncertain future (Vajravelu and Daniel 1983, Pandurangan and Pushpangadan 1997). The geographical distribution of *Impatiens* is very localized and endemic (Tian et al. 2004). In India itself, the species are local.

Though the ideal climatic conditions prevailing in the Western Ghats region provide suitable habitat for the *Impatiens*, their populations are rapidly declining due to various factors such as fragmentation of popula-

tions, narrow environmental niche, habitat degradation, lack of specific pollinating agents, poor seed germination, anthropogenic pressures, reproductive constraints etc. Habitat loss and fragmentation have become great concern to conservation geneticist as anthropogenic activities and environmental deterioration have broken large, continuous population into small and isolated ones. Populations in fragmented habitats are considered more vulnerable to demographic, environmental and genetic stochasticity and therefore, face a higher risk of local extinction (Boyce 1992, Tilman et al. 1994, Lande 1999). Britton (1996) suggested that isolated populations might exhibit high heterozygosity due to strong selection pressure. Such a genetic structure was also detected in populations of narrowly endemic plant established before and after fragmentation (Gonzalez-Astoraga and Nunez-Farfan 2001). Only a few studies have been made by Tara and Namboodiri (1974) on cytology, and by Rust (1977; 1979), Tian et al. (2004) and Sreekala et al. (2007, 2008) on pollination biology of *Impatiens* species due to their high altitude habitats, delicate nature and explosive fruits.

Therefore, it is necessary to understand the biology of a species to find out the causative factors, which lead to reproductive failure and ultimately affect the populations in the wild. Against this background, ones such rare balsam namely *Impatiens dalzellii* Hook. f. (exclusively endemic to Maharashtra state, India) has been selected for the present investigation on phenology, pollination, pollinator behaviour, pollen viability, stigma receptivity and seed germination etc. to find out feasible reason for limited its distribution in the nature.

MATERIALS AND METHODS

The study site lies between 10° 75' - 18° 15' North latitudes and 73° 60' - 76° 40' East longitudes and extends over the range of altitude 1230-1436m. This area experiences both southwest and northwest monsoons and receives an average of 430cm ±20 rainfall. Sometimes rainfall exceeds over 633cm in some areas of Mahabaleshwar. The region harbour vegetation types such as subtropical evergreen, semi-evergreen, montane grasslands etc. which form natural home for many *Impatiens* Linn. species.

Impatiens dalzellii Hook. f. is distributed from Mahabaleshwar and Panchgani (near tableland) of Satara district, Maharashtra State, India. It is an erect succulent herb reaching up to 30-70cm high (Figure

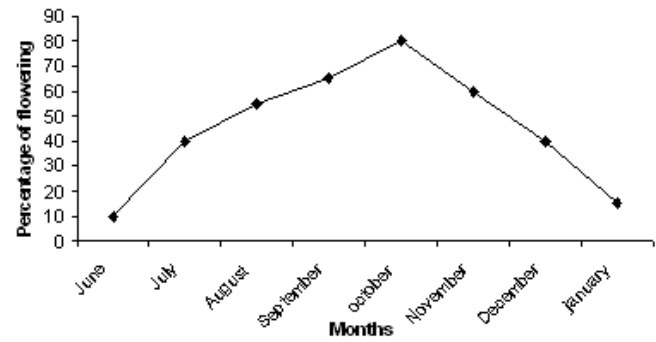
2a), along the road-sides. The yellow colored flowers are hermaphrodite, although they do not reach high fruit or seed set without the co-operation of pollinators. The inflorescence is axillary dichasium-type, within inflorescence variation in flower size, fruit and seed set is very common. Flowers have 3 sepals, often colored, lateral sepal small, lip (lower sepal) resupinate, boat shaped, streaked inside with red nerves; spur very short. Petals 3 showing upper standard and 2 lateral wing petals, yellow (Table 1, Figure 2b). Capsules are ellipsoid and turgid in the middle, tapering at both ends (Figure 2e). Seeds are very few rarely 6-9, shiny black with caruncle near the hilum.

Flowering phenology was observed at plant and inflorescence level with reference to day-to-day flowering pattern in twenty-five marked plants. Fifty flowers were sampled to record the floral morphology and pollen characters. To estimate the amount of nectar, the flowers were selected randomly from different habitats and bagged just before opening of flower to prevent flower visitors. They were excised at hourly intervals (N=20) and the amount of nectar was estimated using a calibrated microcapillary tubes (5µl Drummond microcaps). Anthesis, anther dehiscence and stigma receptivity were studied using various methods as described by Shivanna and Rangaswamy (1992). The number of pollen grains/anther/flower was determined from 25 flower following Cruden (1977). Pollen size was measured with an ocular micrometer under light microscope following the procedure of McKone and Webb (1988). The number of pollen grains divided by the number of ovules per flower will yield the pollen-ovule ratio (Cruden 1977). Pollen viability was assessed by the method suggested by Dafni (1992). *In-vivo* pollen germination was checked by aniline blue fluorescence microscopic method as described by Shivanna and Rangaswamy (1992).

Breeding behaviour (autogamy, geitonogamy and xenogamy) was tested using controlled pollination. In order to observe the rate of natural fruit-set, 60 inflorescence on different plants were tagged and were followed until fruit development. Foraging behaviour of insects was recorded in the field conditions as well as they were captured using an insect net and identified with help of experts from Kerala Agriculture University and our research group. Foraging activity during night was observed using red light. Pollination efficiency of different pollinators was checked by observing the pollen load on different body parts under the microscope according to the procedure given by Kearns and Inouye (1993).

RESULTS AND DISCUSSION

Impatiens dalzellii Hook. f. set full vegetative growth following monsoonal rains in June and start flowering during late July and extended up to December with maximum bloom in October (Figure 1, Table 1). Flowering is synchronous in the habitat. The flowers are born in axillary fasciated racemes. The flower buds take 10-15 days from initiation to full bloom. The flowering days extended up to 180 days in a year and average life span of the individual flower is 2-3 days. The flowers open in the night time between 0200-0315h (Table 1) and extended for 24 hour. The flowers offer both pollen and nectar to the visitors. There is hardly any nectar in the short spur soon after anthesis, but it accumulates gradually and reaches a maximum of about 5 μ l by 1100h. Anther dehiscence by apical pore in the night time one day before flower opening around 0230-0330h (Table 1). This confirmed the protandrous condition of the flowers. Bhaskar and Razi (1974) had reported that majority of the wild balsams grown in the high altitude areas are night blooming and have a wide range of timing with regard to anthesis and pollen germination. Study of anthesis and anther dehiscence is vital for subsequent dispersal of pollen grains into the atmosphere. Pollen grains are spherical, tricolporate with reticulate exine and 28.5 μ m in diameter. The mean number of pollen grains and ovules per flower of *Impatiens dalzellii* is 30,480 and 6 respectively, therefore the ratio of pollen grains to ovule number is 5080:1 (Table 1). Pollen productivity depends upon anther length, pollen grain size and mode of anther dehiscence (Linskens 1992). Total pollen output is related to fruit and seed setting (Bhattacharya and Mandal 2000).

Figure 1. Flowering of *Impatiens dalzellii* in different months.Table 1. Floral characters of *Impatiens dalzellii*

Floral Characters	Observations
Flower colour	Yellow
Flowering period	July-December
Odour	Mild
Nectar	Present (minor)
Flower opening time	0200-0315h
No. of anthers/flower	5
Anther dehiscence time	0230-0330 h
Anther dehiscence mode	Apical pore
Average no. of pollens/anther	6096 \pm 30
Mean no. of pollen grains/flower	30,480 \pm 50
Mean no. of ovules/flower	5
Pollen-ovule ratio	5080:1
Pollen type	3-colporate
Pollen size	\pm 28.5 μ m
Pollen shape	Spherical
Stigma type	Wet and non-papillate

Table 2. List of insects and forage collected by them in *I. dalzellii*

Order	Family	Species	Foraging nature	Foraging time
Hymenoptera	Apidae	<i>Apis florea</i>	N and P*	Day
		<i>Apis indica</i>	N and P	Day
		<i>Trigona iridipennis</i>	P	Day
Diptera	Calliphoridae	<i>Chrysomya megacephala</i>	N	Day
Lepidoptera	Papilionidae	<i>Pachliopta aristolochiae</i>	N	Day
	Danidae	<i>Euploea core</i>	N	Day
	Sphingidae	<i>Macroglossum corythus</i>	N	Night
		<i>Macroglossum heliophila</i>	N	Night

*N and P – Nectar and Pollen

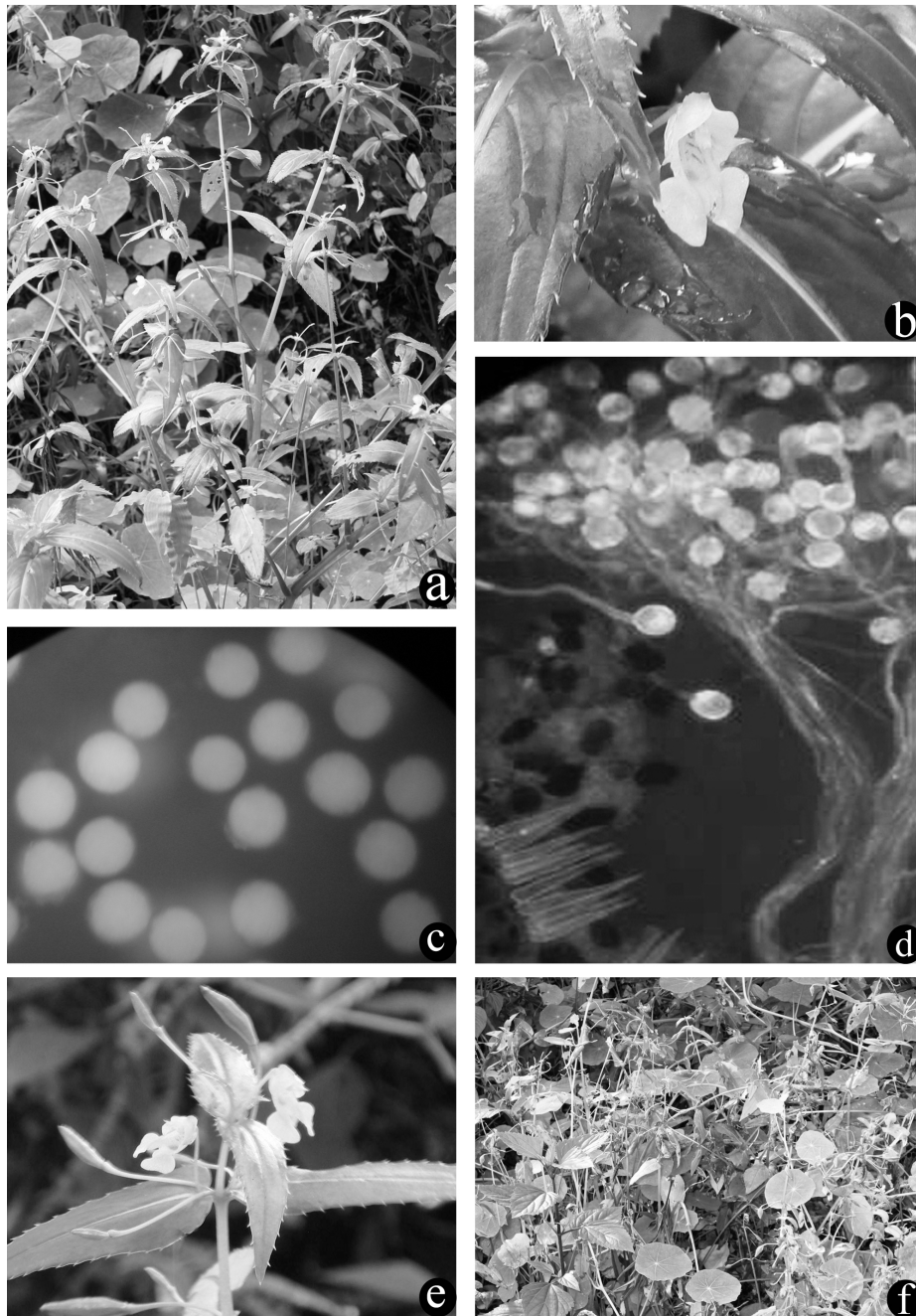


Figure 2. *Impatiens dalzellii*: a) Habit, b) Single flower-front view, c) Pollen viability by FCR test, d) *In vivo* pollen germination using FDA, e) Fruit set through xenogamy (arrow shows elongated capsules), f) Plants growing in association with candidate species.

Pollen viability assessed by FCR (fluorochromatic reaction) test using FDA (fluorescein diacetate) revealed that 45% pollen grains are viable on the day of anther dehiscence and 88% viability on the day of flower opening, showing maximum viability (Figure 2c). The viability gradually reduced on successive days

after anthesis in *Impatiens dalzellii*. Pollen viability is critical for the study of monitoring pollen vigor, genetics and pollen-stigma interactions, crop improvement and breeding programme, gene bank maintenance, incompatibility and fertility studies and evaluation of dispersal and gene flow (Mulugeta et al.

1994, Dafni and Firmage 2000). The stigma is a wet and non-papillate type. It becomes receptive one day after anthesis around 0345h and remains so until the late morning (0800-0930h of the next day). Pollen germination and subsequent post pollination events depends upon the receptivity of the stigma, its nature and compatibility. Receptivity of stigma is a critical factor for successful completion of post pollination events. In *Impatiens dalzellii* the stigma considered receptive when the lobes becomes star shaped showing maximum activity of enzymes such as esterases, dehydrogenases, peroxidases, galactosidases etc. during peak receptivity period.

In-vitro pollen germination studies indicate that 70% of pollen grains were germinated and achieved tube length of $798\mu\text{m} \pm 5$ in 5% sucrose medium. Germination percentages are very low in higher concentrations of sucrose medium. Best pollen germination (83%) along with $872\mu\text{m}$ tube development occurred in Brewbakers medium after 2 hours of incubation. Sucrose is the best carbohydrate source for pollen germination, as it maintains the osmotic pressure of the medium and acts as a substrate for pollen metabolism (Johri and Vasil 1961, Shivanna and Johri 1985). It is believed that pollen of *Impatiens dalzellii* requires more respiratory substrates like sucrose and also requires boron and calcium for its germination and tube extrusion. In Brewbakers medium boron is a stimulatory agent for pollen germination and tube elongation. It is involved with translocation and metabolism of sucrose. The formation of protrusions through germ pore is independent of boric acid supply but tube growth depends on upon the presence of boric acid. Regarding the effect calcium on pollen tube growth. It is said that calcium is concerned with the crowding effect of pollen grains. The crowding effect or population effect is found in the pollen of many taxa (Shivanna and Johri 1985). According to Brewbaker and Mazumdar (1961), this population effect is due to a heat-stable substance called pollen growth factor (PGF) and it was found to be calcium ion (Brewbaker and Kwack 1963). These observations are similar to that of *Impatiens reptans* Moon in China (Tian et al. 2004).

The stigmas were showing 42% *in-vivo* pollen germination along with $612\mu\text{m} \pm 15$ long pollen tube on stigmatic surface (Figure 2d). Subsequently *in-vivo* germinating pollens were decreased on successive days after flower opening. In *Impatiens dalzellii* pollen grains well adhered on the stigmatic surface and pollen tubes penetrate the stigmatic region, reached up to the ovary

and fertilized the ovules. Different breeding experiments were carried in the field to find out the reproductive capacity of the candidate species. Flowers are cross-pollinated as confirmed by various hand-pollination experiments. The fruit set percentage through xenogamy is 75 and there is no fruit set by other modes of pollination. The fruit development took 12-15 days for attaining maturity after fertilization. In the present investigation artificial cross pollination especially through xenogamy enhanced the rate of fruit set in *Impatiens dalzellii*. After maturation, the fruit wall ruptured and the seeds were ejected from the fruits at a distance of 1-1.5m. Each capsule contains 5-8 seeds. The seed germination percentage in natural habitat was limited to only 20.

It is well known that the flowers of *Impatiens* Linn. have enormous diversity and different pollinators. In this study, bees (*Apis florea* Fabricius, *Apis indica* Fabricius and *Trigona iridipennis* Smith.), moths (*Macroglossum corythus* Walker and *Macroglossum heliophila* Boisduval), butterflies (*Pachliopta aristolochiae* Fabricius and *Euploea core* Cramer) and flies (*Chrysomya megacephala* Fabricius.) etc were pollinated the *Impatiens dalzellii* growing on a mountain in the Mahabaleshwar of Western Ghats, India (Table 2). They visit the flowers between 0600 and 1130h for nectar and/or pollen except moths which are night pollinators visited between 2330-0230h. Recently Tian et al. (2004) in *Impatiens reptans* Moon from China and Sreekala et al. (2007; 2008) in *Impatiens henslowiana* Arn. and *Impatiens coelotropis* Fischer from India noticed these night pollinators (moths) in their findings. Therefore, present observations correlate with earlier investigations. In subtropical regions of Africa the *Impatiens* are pollinated by humming birds as well as by insects (Grey-Wilson 1980). In temperate zones, pollinators are bumble-bees and humming birds only (Rust 1977, 1979, Heinrich 1979 and Kato et al. 1989). Hence, in different climatic regions, species of pollinators vary with respect to geographic set-up. Among honey bees *Apis florea* and *Apis indica* forage during 0630-1100h, where as *Trigona* sp. during 0700-0900h which foraged only pollens and hoarded in pollen baskets. Among moths (*Macroglossum corythus* and *Macroglossum heliophila*) are the intermittent visitors, they foraged only nectar during their visit. When the weather was fine, butterflies (*Pachliopta aristolochiae* and *Euploea core*) visited the flowers, spending an average of 2-4 seconds on individual flowers and collected only nectar throughout their trip. Of the total visits, honey bees made 50%, *Trigona iridipennis* 20%, moths 10%, flies

10% and butterflies 10%. Butterflies have not been considered as pollinators of *Impatiens* in Japan and Sumatra (Tian et al. 2004). The effect of nectar gathering on visitations by pollinators was significant. After nectar was gathered from an individual flower, it was not visited by a pollinator until the volume of nectar had returned to a level to attract pollinators. Temeles and Pan (2002) have suggested that nectar gathering did not affect seed set but this conclusion was drawn during peak flowering. It was observed that, although there were hardly any pollinators present during the late phase of flowering, there were abundant nectar gatherers. Hence, it is suggested that nectar gathering might hasten the end of flowering.

Pollination system may be seen as a biological market. Differences in the quality of the 'output' (e.g. nectar quantity and concentration) offered by the 'seller' (plant species) attracted different 'customers' (nectar gatherers and pollinators) (Campbell and Motten 1985). Chittka and Schurkens (2001) reported that, after it had invaded Europe, *Impatiens glandulifera* Royle (Himalayan species) tempted bee pollinators away from native plants as it secreted much more nectar than any of the native species. It was found that, at the end of the flowering period of *Impatiens dalzellii*, some entomophilous plants such as *Asystasia gangetica* (L.) T. Anderson, *Strobilanthus barbatus* Nees. (Acanthaceae), *Viola pilosa* Blume. (Violaceae), *Tropaeolum majus* Linn. (Tropaeolaceae) and *Begonia integrifolia* Dalz. (Begoniaceae) entered their peak flowering phase and secreted significant more nectar than *Impatiens dalzellii* (Figure. 2f). Hence the pollinators that previously visited the flowers of *Impatiens dalzellii* were attracted to visit the flowers of abundant nectar producers. The views of Heinrich and Raven (1972) and Heinrich (1975; 1979) shows that it is the principle aim of insect pollinators to forage nectar as a source of energy and that they will select plants that secrete most, is supported the present observations, reported here. Plants of *Impatiens dalzellii* occurred mostly along the road side or in damp places or as a number of individuals within a group. Isolated individuals were rarely found. Although endemic to Maharashtra of Western Ghats, environmental niches suitable for the establishment of *Impatiens dalzellii*.

The seeds were ejected from the mature capsule to a distance of only 0.5m to 1.5m. Most seeds were dispersed around the maternal plants. Even though some seeds were deposited into the streams and swept downstream, the percentage germination of these seeds was very low. Even if the seeds were able to germinate

in new habitats and develop into seedlings, they were often unable to compete with associated species in the struggle for survival. Hence the spread of the species is limited. Habitat disjunction leads to lower population persistence (Ruggiero et al. 1994). *Impatiens dalzellii* grows in the rural areas along the road side and pastures. With over-exploitation of agricultural resources, and improper management of forest ecosystems, villagers and tourists disturb the original habitats of *Impatiens dalzellii*. Under such conditions, weeds such as *Alternanthera tenella* Colla. and other grass species compete for resources with *Impatiens dalzellii* and spread rapidly, posing a direct threat to the growth of *Impatiens dalzellii*. If habitats continue to be destroyed, populations of *Impatiens dalzellii* the only representative of yellow color flower in the Western Ghats, will continue to decline. It is therefore concluded that all of the above factors either alone or in-combination are responsible for its rarity and limited distribution in the wild habitat.

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