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Pollination Biology of *Rhododendron macabeanum* Watt *ex* Balfour *f.* of Ericaceae in Nagaland, India

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Abstract

The pollination biology, breeding system including pollen-ovule ratio, fruit and seed-set and viability of seeds in some marked plants of *Rhododendron macabeanum* Watt ex Balfour *f.* were made at Khonoma Dzukou, Kohima District of Nagaland (North-East India) at an altitude ranging between 2500 – 2700 m amsl. The flowers which are present in trusses of 20 – 28 flowers are yellow with a purple blotch on the throat are foraged and get pollinated by the birds of the genus *Yuhina*. The other foragers like beetles and flies are insignificant pollinators of this taxon. The only attractant for the foragers is nectar which is secreted in the five nectaries present at the base of the corolla tube. The pollens are present in tetrads which are held together by viscin threads. The flowers of *R. macabeanum* are self-compatible and the plants show high percentage of fruit-set in open pollinated flowers. The climatic conditions like rain and sloppy habitat have been identified the vital factors for poor seedling establishment in the natural locality and hence, are also responsible for the depletion of population of this important taxon which is endemic to Nagaland and Manipur states of North-east India.

Key words: Climatic factor, Khonoma Dzukou, Pollination, *Rhododendron macabeanum*, seedling establishment, Y*uhina* sp.

INRODUCTION

The genus *Rhododendron* Linnaeus is one of the largest genus of family Ericaceae with around 1200 species (Rotherham 1983). Cullen (1980) has also reported *Rhododendron* as largest genus of Ericaceae in China and Himalayas. According to Brown *et al.* (2006) various species of *Rhododendron* are widely distributed between latitudes 80° N and 20° S and are considered Alpine native plants from North America to Europe, Russia, Asia and from Greenland to Queensland, Australia and the Solomon Islands. For its beautiful flowers and foliage the genus has immense horticultural importance (Mao & Gogoi 2012) and the only genus in the family that reaches the height of a tree (Milleville 2002). In the Indian Himalayan Region a total of 87 species, 12 sub-species and 8 varieties of rhododendrons have been recorded by Sekar & Srivastava (2010) out of which 20 taxa are endemic, 30 rare, 24 threatened/endangered, 3 vulnerable and 47 taxa are yet to be assessed. The major threats to rhododendrons are deforestation and unsustainable extraction for firewood and incense by local people.

Reproductive biology of Rhododendrons has been studied by Ng & Corlett (2000), Mejias *et al*. (2002), Ono *et al.* (2008), Escaravage *et al*. (2011), Williams *et al*. (2011) and Ling (2011). However, Moza & Bhatnagar (2007) have emphasized the importance of the studies of reproductive biology of RET taxa in determining the factor/s responsible for the depletion of their population in the natural habitat. They suggested that such studies will be useful in the sustainable development as well as conservation of RET taxa. Keeping these points in mind the present studies on the reproductive phenology of *Rhododendron macabeanum* Watt *ex* Balfour *f*., a Rare and Endangered species which is endemic to Nagaland and Manipur (Sastry & Hajra 1983; Paul *et al*. 2005; Mao 2010; Sekar & Srivastava 2010; Mao *et al.* 2011; Mao & Gogoi 2012), has been undertaken.

The present paper deals with the reproductive phenology which includes pollination biology as well as seed and fruit set in *R. macabeanum.* The outcome of present studies will provide valuable information for the appropriate conservation and propagation of *R. macabeanum*.

MATERIALS AND METHODS

The present investigation was carried out during 2012 – 2013 on 20 marked plants of *Rhododendron macabeanum* Watt *ex* Balfour *f.* growing in the natural populations at Khonoma Dzukou, situated at 25°36′ N, 93°59′ E and at the altitude of 2500 – 2700 m asl. Plants were observed daily during its entire flowering period (March & April) to study the flowering period and pollination biology. Flower longevity was determined by marking 50 buds on 50 branches in each marked plant following Gill *et al*. (1998).

Periodic flowering, of *R. macabeanum*, during initiation, peak, and end as well as the relative flowering intensity (average number of flowers / inflorescence x average number of inflorescence / individual) was registered from 20 marked plants following Dafni (1992). The periodic observations on anther dehiscence and stigma receptivity were recorded carefully.

The number of pollen grains present per anther as well as per flower was counted by teasing out the mature anthers (10 flowers per plant) in lactophenol-glycerine with aniline blue. A known dilution was placed on the grid and 10 replicate counts were made using a haemocytometer as suggested by Barret (1985). Pollen viability of all the marked plants was checked with 0.2 % TTC solution (2, 3, 5-triphenyl tetrazolium chloride) following Hauser & Morrison (1964). Number of ovules per flower (20 flowers per marked plant) was recorded following Stelly *et al.* (1984). The number of ovules was counted using Leica digital stereooom microscope. Pollen-ovule ratio was determined by the method after Cruden (1977). Seed viability was tested by triphenyl tetrazolium chloride (TTC) test as described by Kearns & Inouye (1993).The photomicrographs were taken under Leica- 1000 microscope and the field photographs were taken with the help of Canon still digital Camera model- EOS 550D.

RESULTS AND DISCUSSION

Rhododendron macabeanum is a large tree growing to a height of above 12 – 15 m. At the branch tips 20 – 28 lemon yellow flowers are produced per truss (Fig.1A). The bark is reddish brown and peels off easily. Leaves are broadly ovate to elliptic. The species is usually found growing on the rocky hill-slopes in association with some other species of the genus, such as *R. maddenii* Hooker *f.*, *R. elliottii* Watt and *R. triflorum* var. *bauhiniiflorum* (Watt ex Hutchinson) Cullen. Also, plants like dwarf bamboos, Berberis and certain fern species are common in the habitat.

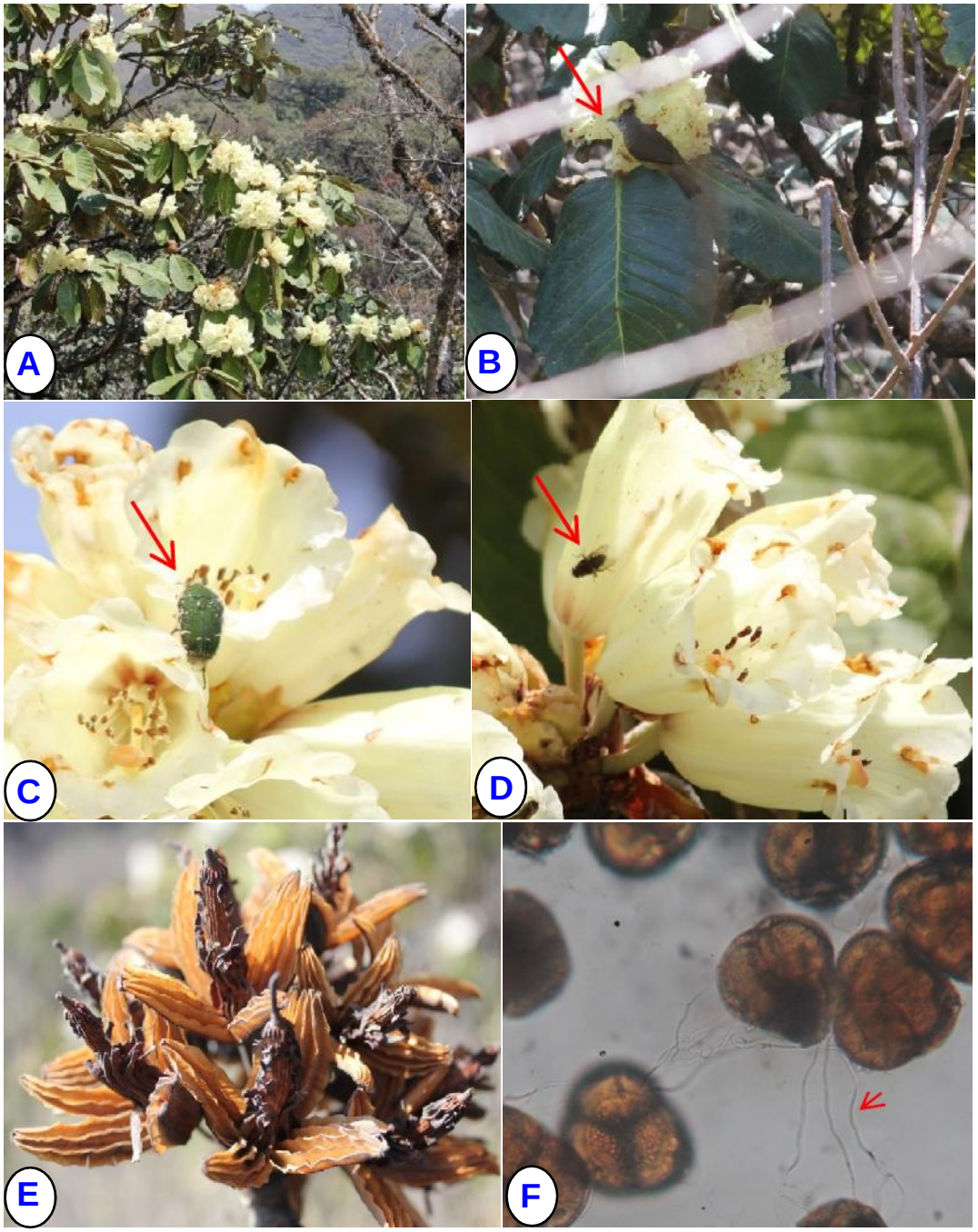

PLATE - I. Figs. A - F. *Rhododendron macabeanum* Watt *ex* Balfour *f.* [Ericaceae] showing floral biology: **A.** Flowers in full bloom;**B.** A bird *(Yuhina* sp.) foraging on the flowers; **C.** A beetle visiting the flower; **D.** A fly foraging the flowers for nectar as well as pollens; **E.** Dehisced fruits; **F.** Pollen tetrads with viscin threads. [Arrows in the photographs indicate the objects as mentioned]

Floral morphology and flowering phenology: The initiation of floral buds takes place in the month of November. The blooming takes place in the first week of March. The peak of the blooming was observed in the last week of March which becomes over by the end of April. Inflorescence is ball shaped, dense, 20 – 28 flowered; corolla 8-lobed, tubular-campanulate, lemon yellow with a purple blotch at the base. Generally 16 unequal stamens are present in a flower; anthers dorsifixed , lobes brown, dehisces through apical pores. Ovary conical, densely rufous-tomentose; stigma cup-shaped, red on a persistent style covered with stalked glands and hairs. The stigmatic groove secretes sticky exudates (nectar).

Pollen biology: The pollen grains are present in tetrads and are held together by viscin threads (Fig.1F). The pollen tetrads come out of anthers by vibration o shaking caused by the wind as well as the floral visitors. The anthers dehisce through the apical pores before the flowers open (Protandrous condition, i.e. stigma become receptive after the anther dehiscence of the flower). Each pollen grain is tricolporate. The similar structure of pollen grains has also been described by Williams *et al.* (2011) for the pollen grains of several other species of the genus *Rhododendron.*

Table 1. Depicting the pollen: ovule and ovule: seed ratio in *Rhododendron macabeanum*

No. of pollen tetrads per flower	Average no. of pollen tetrads per flower	No. of ovules per flower	Average no. of ovules per flower	Pollen : ovule ratio	No. of seeds per flower	Average no. of seeds per flower	Ovule : seed ratio
87223	1,17,617	1920	2,339	50.29	1034	1301	1.80
1,45,872		3208			1470		
1,54,960		1960			980		
99,837		2646			1235		
100,197		1963			1789		

The average pollen production per flower is 1, 17,617 and an average production of ovules per flower has been found as many as 2,339. So, the average pollen-ovule ratio becomes 50: 1 (Table-1).

Pollination biology: The lemon-yellow flowers of *R. macabeanum* are foraged by birds of the genus *Yuhina* for nectar (Fig. 1B) by inserting their head within the corolla tube. Pollen tetrads were seen attached on their beak and with the feathers of dorsal as well as the ventral surfaces during sunny days between 9.00 AM to 2.00 PM. The duration of foraging visit lasts for 1 – 3 minutes. The Beetles and flies also visit the flowers (Fig.1C, D) but their contribution in pollination cannot be ascertained. However, no visitor was observed during misty, windy and rainy weather.

Fruit and seed-set: There are 20 – 28 flowers in one inflorescence and an average of 20.71 fruits are produced in each infructescence and the fruit set percentage is 71.43 %. Capsules grooved, rufous-tomentose, 16 – 18 chambered and dehisced by longitudinal slits (Fig.1E). The seeds are small, reddish brown and dispersed by wind. The average number of seeds per fruit is recorded as 1301 (Table 1). Ovule-seed ratio is recorded as 2: 1. *In vitro* seed germination was 80 % but seedling survivability was only 10 %.

Breeding system: Fruit set percentage in the natural population is 71.43 %. *R. macabeanum* is self-compatible and exhibit facultative geitonogamy as reported by Cruden (1977) for many other species of flowering plants.

CONCLUSION

The main pollinators of *Rhododendron macabeanum* Watt *ex* Balfour were found to be the birds of genus *Yuhina* as they forage for nectar and carry pollen on their beak, fore-head as well as on the ventral and dorsal surface of the neck. The other visitors like beetles and flies have not been found to be effective pollinators. The flowers are self-compatible and also exhibit geitonogamy and xenogamy due to frequent movement of these birds from one flower to another in search of nectar. The plants show high percentage (71.43%) of fruit set in open pollinated flowers. The pollen-ovule ratio has been found as 50.29: 1 (or, 50: 1) and the ovule: seed ratio has been found as 1.8: 1 (or, 2: 1) and pollination experiments indicate that it is self-compatible and facultative geitonogamous as suggested by Cruden (1977). Although the seed-set percentage has also been very high, yet the seedling establishment in natural habitat is very low. Since, the seed germination in the laboratory has been found 100 %, the poor seedling establishment in the natural habitat has been assigned to the various climatic factors like heavy rains and the sloppy habitat around the population of *R. macabeanum* trees which could not provide the proper conditions for seedling establishment into the soil after the seed germination.

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