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Reproductive biology of *Elaeocarpus blascoi* Weibel, an endemic and endangered tree species of Palni Hills, Western Ghats, India

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Elaeocarpus blascoi Weibel is a lesser known endemic tree species growing in the Vattakanal shola forest of Palni Hills, Western Ghats, India. A study on reproductive biology of the species was conducted in the natural habitat to study its phenology, floral biology, pollen biology, fruit set and seed germination. Flowers are bisexual, anther dehisces 2–3 hours after anthesis and stigma becomes receptive on the day of anthesis and extends up to 6 days. Breeding experiments confirmed that the species permits autogamy and geitonogamy. Six different pollinators were observed during peak flowering period and *Apis dorsata* (honey bee) was found to be an effective pollinator and it takes 55 ± 15 sec per flower. Percentage of fruit set observed in the natural habitat was 78% and seed germination rate was found to be less than 5% in the natural habitat. Tests showed that more than 70% of seeds lost their viability after a year and most of the seeds were infested with *Fusarium* sp., *Lasiodiplodia* sp. and *Penicillium* sp. Further, the natural habitat of the species is altered by commercial plantations, tourism and urbanization in the Palni Hills, which leads to their reduction.

Keywords: *Elaeocarpus blascoi*, endemic species, reproductive biology, shola forest.

IN Asia, the genus *Elaeocarpus* consists of 120 species of which 25 have been reported from India¹. Most of the species of *Elaeocarpus* are confined to the North East and southern India, and a few species to Andaman and Nicobar Islands. Eleven species were reported from the Western Ghats of Tamil Nadu (TN), including the recently reported *Elaeocarpus aristatus*². The species of *Elaeocarpus*, generally prefer a warm humid climate and usually occurs between 500 and 2000 m amsl (ref. 3). Usually, the genus consists of large trees with buttressed root along the perennial streams of tropical forests, mostly in the evergreen and shola forests. Though it is widely distributed, the species is never found in abundance in any particular locality. The fruits of *Elaeocarpus* species are endowed with a hard and highly ornamental stony endocarp. In nature, the rate of seed germination of most species of *Elaeocarpus* is low and erratic, since the nuts are unable to imbibe water⁴. Poor or no germination

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of seeds coupled with prolonged dormancy owing to hardness of the endocarp cause a significant reduction in the regeneration of several species of *Elaeocarpus*⁵. Of the 12 species reported from the Western Ghats, six are under sever threat of extinction and included in the IUCN red list.

Elaeocarpus blascoi Weibel is a medium-sized shola tree discovered by Blasco in 1970 from Bear shola of Palni Hills, TN, the species was scientifically described by Weibel⁶. It is a canopy tree growing up to 20 m in moist evergreen forests with short, young branches appearing grey with silky hairs and fallen leaf scars⁷. It was found to be extinct later during the exploration of flora of the Palni Hills⁵. After a long gap of 41 years, it has been rediscovered in another region, Vattakanal shola of Kodaikanal, Palni Hills, with a single surviving individual⁶. It is a strict endemic species to Palni Hills of Western Ghats and found on the fringes of the moist evergreen forest at 2011 m amsl and included under 'endangered' category by IUCN⁸. There is confusion among researchers on the identification of *E. blascoi* and *E. munroii*. The former closely resembles the latter by having terminal or axillary racemes, crowded leaves at the end of the branches and small seeds, but it shows great variation in leaf, flower and fruit morphology. *E. munroii* trees grow up to 40 m having subcoriaceous leaves, tender parts are glabrous and fruits oblong, whereas *E. blascoi* grows up to 20 m with leaves being coriaceous, tender parts sericeous and fruits ellipsoidal. After periodical field exploration by several researchers and also by our team, it has been concluded that only a single individual seems to be surviving at present in the forest areas of Vattakanal shola, Kodaikanal. Also, a medium-sized adult flowering tree has been planted on the roadside along with a small tree conserved in the NGO garden at Kodaikanal. Thus a total of only three individuals of *E. blascoi* are surviving at present in the world⁷. After the rediscovery of *E. blascoi*, no effective conservation strategies have been undertaken to increase their numbers, and this shows that the tree has been facing many problems in its regeneration.

The study on reproductive biology of flowering plants is an important aspect for determining the barriers to set fruit and seed, and also for understanding pollination and breeding systems that regulate the genetic structure of plant population⁹. Any intervention between flowering to fruit set and seed germination will cause a drastic reduction in the production of offspring and also in the population of trees in their natural habitat in the near future. Hence, the reproductive biological studies have gained importance, especially for species which are on the verge of extinction. The resulting data from the study can be utilized for framing better conservation strategies and for proper management of the tree species in their natural habitat. Further, the study will also provide adequate knowledge on plant–animal interactions during pollina-

tion, seed dispersal and distribution of the tree population in its natural habitat.

There is a widespread consensus on the reproductive biology of endangered, rare or threatened species, and this may be useful for understanding why they are endangered, rare or threatened¹⁰. Information obtained from reproductive biological and conservation studies may be useful for evaluating alternative *in situ* and *ex situ* management strategies¹¹. Endemic species with restricted geographic distribution have become a central concern of biologists faced with the problem of preserving rare and endangered species due to habitat destruction and fragmentation. Among the endemic species most prone to such effects are those in which the population size is small¹². Although many important investigations on reproductive ecology of tropical trees have been undertaken and most species of trees are remain uninvestigated^{13–15}. Hence, the present study is aimed to investigate the reproductive biology of two flowering individuals of *E. blascoi* at two different sites of Palni Hills to understand their reproductive inefficiency and constrains for limited distribution.

Palni Hills is a part of the Southern Western Ghats and extends as an eastward spur with tropical forest. The prevalence of tropical climate conditions and high rainfall makes it home to many endemic species along with few strict endemics. Palni Hills is located at the middle of the Western Ghats in TN and receives maximum rainfall during the northeast monsoon and a least amount during the southwest monsoon. Further, the region may also receive rainfall occasionally during summer. It receives an average rainfall of 1650 mm and prevailing temperature ranges from 8° to 20°C. The study on reproductive biology of *E. blascoi* was undertaken in the forest areas of Vattakanal shola (10°12'29.6°N long. and 77°28'50.3°E lat.) in Kodaikanal. This region mostly consists of evergreen tree species adjacent to the grasslands commonly called as sholas or tropical montane forest. *E. blascoi* is a medium sized shola tree with buttressed root found on the fringes of the evergreen shola of Kodaikanal Hills at an altitude of 2011 m. The species is represented by the single individual in the wild, found along with some shola arboreals like *Szygium densiflorum*, *Rhododendron arboreum*, *Elaeocarpus variabilis*, etc. The present study was carried out on approximately 45–57 years old *E. blascoi* tree from 2012–2014. However, an adult tree planted on the roadside was also observed for comparative studies on reproductive biology.

The trees were periodically monitored for different phenological events covering leaf flushing, flowering, anthesis, fruit initiation and development, and seedling establishment in the natural habitat. Seed germination was also observed in the natural habitat during the monsoon season. Flowering phenology was observed on a day to day basis in the natural habitat according to the method suggested by Dafni *et al.*¹⁶. To estimate the reproductive

ability, flower–fruit ratio was calculated by randomly selecting 100 flowers and was allowing them to set fruit; the ovule–seed ratio was also calculated. The initiation, development and maturation of inflorescence and flowers were observed on a daily basis during the flowering season and the number of flowers produced per inflorescence, lifespan of flowers, time of anthesis, time of anther dehiscence, nectar production and stigma receptivity were recorded by continuous observation. Further, the morphological characters of flowers were analysed by collecting them at different stages and observing them under a dissection microscope. The observations were based upon the spatial and temporal arrangement of mature male and female sexual parts within the flowers. Morphology of the pollen grains was studied with an ocular-stage micrometer under light microscope using acetolysis method. Pollen–ovule ratio was worked out according to the method suggested by Cruden¹⁷. Pollen viability was assessed by 2,3,5-triphenyl tetrazolium chloride, di amino benzidine¹⁶ and fluoro-chromatic reaction test¹⁸. Pollen germination was studied *in vitro* with different concentrations of sucrose in modified Brewbaker's medium in order to analyse the viability at different time periods. The male maturity was assessed at pollen maturation and anther dehiscence, and female maturity assessed at stigma receptivity in which bubbles of oxygen evolve from the stigma when submerged in hydrogen peroxide¹⁹.

During the flowering period in general and at the time of anthesis in particular, the tree was continuously monitored during day and night to record the different pollinators visiting the flowers. The foraging behaviour of floral visitors was analysed by photography and visual observation using high-resolution binoculars (Olympus). Pollination efficiency of different pollinators was checked by observing the viable pollen load on different body parts under a microscope according to the procedure given by Kearns and Inouye¹⁹. After each visit of the pollinators, the marked stigmas were observed carefully by hand lens (40x) and the viable pollen transfer to the stigma was confirmed by various tests. The foraging period and type of food collected by different pollinators/visitors were also recorded by close observation. Several aspects of pollinator behaviour viz. contact with the male and female parts of the flower, length of visit, foraging and the number of flowers visited by the individual pollinators were recorded.

Hundred flowers were randomly selected from different inflorescences of two trees growing at different sites and marked for breeding experiments. Twenty-five flowers were bagged properly with butter paper and cotton cloth to prevent the deposition of foreign pollen grains and to confirm self-compatibility. Fifty flowers were emasculated before anther dehiscence and manually pollinated for geitonogamy and xenogamy experiments and ten flowers were emasculated and bagged properly to confirm the apomixis, if any.

The study on reproductive biology helps in developing strategies to preserve genetic potential of rare species which are crucial for restoration programmes²⁰. Based upon the information, the present communication reports a comprehensive study on the reproductive biology of *E. blascoi*. Since most of the species of this genus are in the threatened categories, the results may be utilized for the development of better conservation strategies for other species in this genus.

Knowledge on phenology and floral morphology is essential for conducting studies on breeding systems, particularly on pollination syndrome, if any. Like many tropical evergreen trees, *E. blascoi* also shows periodic phenological events in relation to seasonal changes. Based upon the phenological investigation and information collected from the local people on *E. blascoi* it can be confirmed that the tree requires approximately 15–18 years for its first flowering. A complete study on the phenophase events reveals that the tree exhibits periodic flowering and fruiting events annually. Leaf flushing (Figure 1a) occurs along with the development of fruit from February and extends up to March. The young shoots and leaves emerge slowly, and new branches and

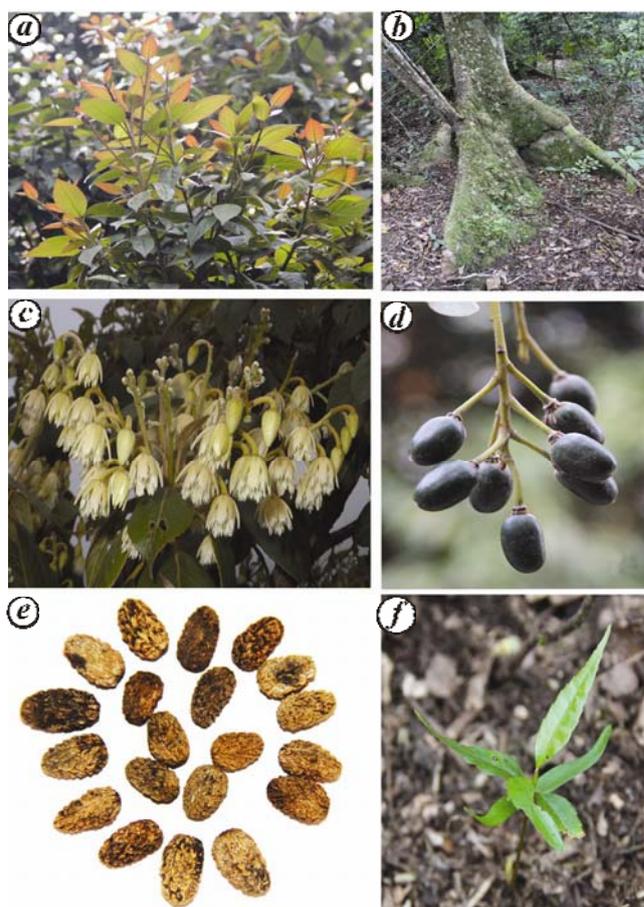


Figure 1.

buttress roots also develop (Figure 1 b) as in other species of *Elaeocarpus*. The younger leaves form a light green to reddish crown on top of the each branch and canopy, which helps differentiate this tree from other shola arbo-reals. There is no mass leaf shedding, as it is an evergreen shola tree. The flower buds emerge in every leaf axis of terminal branches in the first week of September along with the onset of monsoon. Flower buds development occurs slowly; the buds remain unopened for a month and anthesis of first flower occurs in the first week of October. Till the end of December, new floral buds are initiated in the terminal branches. The peak flowering (Figure 1 c) period is observed from October to December where more than 60% of flowers is opened. Flowering period usually ends in January; however, it may sometimes extend up to March.

The development of fruit starts from February and fruit maturation takes place towards the end of May (Figure 1 d). During fruit development, major part of the immature fruits is eaten by unknown larva, which trigger premature fruit fall. Most of the mature fruits are found along with some persistent anthers at the base of the fruit. The fully ripened fruit appears as black-green and the tree sheds fruits from June onwards. The size of the fruit is about 1–2 cm, single seeded and endowed with hard endocarp that makes the seeds to dormant for 12 to 16 months (Figure 1 e). The new seedlings start to develop in September and show slow growth (Figure 1 f). During seedling development, most of the seedlings were found to be infested with fungus (*Rhizoctonia* sp.) and also eaten of by the insects in the natural habitat; thus only a few seedlings were successfully developed after a month.

E. blascoi produces 08 ± 4 inflorescences per twig and each inflorescence bears 7 ± 3 flowers. The bud emerged during September–October produces more flowers (8–10 flowers per inflorescence) and buds emerging after November produce less number of flowers (4–7). The flowers are pendulous and borne on raceme as like other *Elaeocarpus* species. Usually, the older flower parts do not persist until fruit formation. Most of the floral parts were found to be infected with unknown larva even at the bud stage. The floral buds are silky and fully covered with single-celled hairs (Figure 2 a). The anthesis of flowers occurs in the morning between 0700 and 1100 h, and the sepals split longitudinally at one day before anthesis. The diurnal anthesis was also recorded in *E. munroii* and *E. serratus*, but in *E. tuberculatus* the flowers were recorded with nocturnal anthesis²¹. The completely opened flower appears as pale white and silky with a mild odour. The flowers remain fresh for 8 days after anthesis. This result is more or less similar to *E. photiniaefolius*, that was reported to have 6.2 days of flower longevity²². Each flower consists of a maximum 30 anthers, and filaments attached to the base of the ovary with the nectar glands (Figure 2 b). There are ten segments of nectar glands, each pair of segment of nectar gland consist of six

anthers. Shiny yellow appearance of the nectar glands shows the production of nectars. Nectar production was measured as 1.3–1.8 μ l per flower/day. The nectar production was comparatively higher than *E. photiniaefolius*²². The anther opens by apical split and dehiscence 2–3 h after anthesis. Average number of pollen grains per anther was calculated as $50,700 \pm 456$ and per flower was $1,368,900 \pm 2680$. Mean number of ovules per flower was recorded as 24 ± 3 and the pollen–ovule ratio was calculated as 57,031 : 1. Pollen productivity depends on anther length, pollen size and mode of anther dehiscence^{23,24}. Pollen size was measured as 10–12 μ m and found as a cluster due to its sticky nature. *In vitro* pollen germination observed in modified Brewbaker's medium with 20% sucrose showed, 60% of pollen germination after 16 h. Pollen viability by different experiments showed that 77% of pollen is viable at the time of anther dehiscence (Figure 2 c). Ovary was tricarpeillary, fully covered with single-celled hairs and style was longer than anthers. The morphological studies on stigmatic surface of the flowers showed it to be wet and papillate. Stigma receptivity is a critical factor for successful completion of post-pollination events; usually it is highest soon after anthesis, but varies

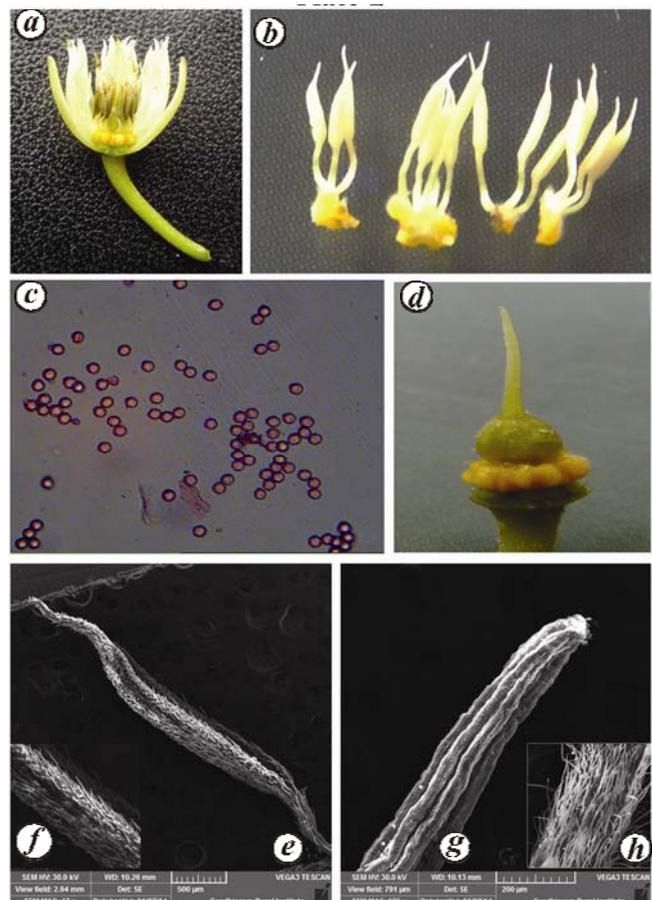


Figure 2.

from species to species depending upon the temperature and humidity²⁵. In *E. blascoi*, the stigma become receptive on the day of anthesis and maintains its receptivity up to 6 days after anthesis (Figure 2 d). This prolonged receptivity period of the species helps maintain their sexual period as balanced. The morphological features of the stigmatic surface (Figure 2 e–h) are as in the other species of *Elaeocarpus*.

Pollinators were found to be active from 0800 to 1500 h. Six different pollinators, viz. honey bees (*Apis dorsata* and *Apis cerana indica*), three species of flies (*Phaenicia sericata* and two unknown flies deposited for identification) and mite (one species) were observed as pollinators during the peak flowering period. *A. dorsata* was found to be a more effective pollinator by transferring viable pollen grain from anther to stigma. *A. dorsata* takes 55 ± 15 sec per flower and regularly visits, the flower in an order from older to the younger flowers in each inflorescence (Figure 3 a). *A. dorsata* was also recorded as pollinator in *E. munroi*²¹, in which it transfers the pollen grains effectively and successfully pollinating the flowers. *A. cerana indica* is found to be active after 1000 h in morning and spends 50 ± 10 sec per flower

(Figure 3 c and e). Honey bees are most active during the morning hours visiting all the flowers in an inflorescences from the old to the newly anthesised flowers and spending more time in each flower (Table 1 and Figure 4). They visit the flowers regularly and each flower is visited again by the same honey bees. Tiny fruit flies and *Phaenicia sericata* are also effective pollinators, they spend 45 ± 10 sec per flower (Figure 3 b and d). *P. sericata* has also been reported as floral visitor in *E. serratus*²¹, in which it is transfers pollen from anther to stigma during the receptive period of the flower. *A. dorsata* visits almost all the opened flowers in each inflorescence and the flies also found to be occasional pollinators of the flowers (Figure 3 f–h). Mite pollinate the flowers in the absence of other pollinators in the late flowering seasons. The mechanisms of flower opening and subsequent anther dehiscence at every inflorescence need floral visitors. The inner floral movement of pollinator towards the nectar glands makes them to trigger the arista (a special projection found at the top of the anther) that causes the apical split of anther to dehiscence and release the pollen grains. Naturally, the flower bends downwards; the stigma is completely closed by the floral parts to receive its own pollen grains from its anthers. The position of the nectar gland being attached at the base of the anther and the ovary makes the pollinators enter deep inside the flower and trigger the anther to release the pollen grains. This mechanism helps in the effective transfer of pollen grains within and among the flowers. On the other hand, the position of flower, spatial arrangement of stigma and anther with apical opening makes the stigma receive its own pollen grains for self-pollination. Hence the morphological features of the flower show that it performs self-pollination. However, the species requires floral visitors



Figure 3.

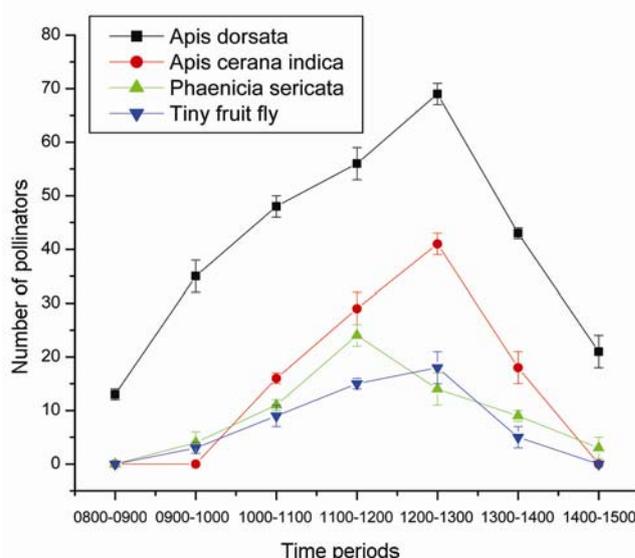


Figure 4. Pollinators and foraging time period on the flowers of *E. blascoi*.

Table 1. Pollinators and their foraging behavior in *Elaeocarpus blascoi*

Pollinators	Visiting time	Foraging period	Time spend/flower	Stigma touch	Reward for pollination
<i>Apis dorsata</i>	Day	08.00–15.00 h	55 ± 15 sec	+++	Nectar
<i>Apis cerana indica</i>	Day	10.00–13.00 h	53 ± 10 sec	+++	Nectar
Carrion fly	Day	10.00–15.00 h	30 ± 10 sec	++	Nectar/pollen grains
Unidentified fly1	Day	09.00–14.00 h	25 ± 5 sec	+	Nectar.
Tiny fruit fly	Day	Permanent during flowering	10 ± 5 sec	+	
Mites	Day	Permanent and found during late flowering season	Present inside the flower	++	Pollen/nectar

+++ , Very good; ++, Good and +, Poor.

**Figure 5.**

to trigger the anthesis of flowers to dehisce the anthers and release the pollen grains. The sticky pollen is transferred from the abdominal hairs and hind legs of the pollinators and gets in contact with the stigma of other flowers during foraging. The flower provides both nectar and pollen as pollination reward. Flies are also found as floral visitors in some flowers and visit one or two flowers in an inflorescence. The floral features such as diurnal anthesis, nectar production, position of anther and stigma allow the pollinators to transfer the pollen grains to other flowers for cross-pollination between the flowers.

Breeding experiments were conducted on randomly selected flowers, where the flowers allowed for natural

pollination showed 78% fruit set, of which 50–55% of the fruits had detached from the mother plant before attaining maturity. The main reason for the premature fruit fall is due to the larval infection in most parts of the tree including tender shoots (Figure 5a and b). Fruit development was slow and took nearly 40–60 days from initiation to maturity and for detachment. On the other hand, fruit set observed in self-pollination (autogamy) was 72% and cross-pollination (xenogamy) showed no fruit set during the study. The bagging experiments confirmed that the tree allows autogamy and geitonogamy, and it is self-incompatible to cross-pollination. Pollination and experiments on flowers for the confirmation of apomixis also failed to set fruits. This shows that the tree does not allow apomixis and xenogamy. Selfing may have selective advantages over outcrossing during colonization or population bottlenecks when access to mate is limited or when outcross pollen is limited because pollinators are scarce or unreliable²⁶. The tree is found to be a single individual and it has to largely depend on self-pollination, the position of the flowers also favours self-pollination.

The natural fruit set was recorded as 78% and manual pollination did not considerably increase the fruit set rate. The matured fruits yield viable seeds with less than 5% of germinability which causes the reduction in seedling establishment in the wild. The seeds are endowed with hard endocarp which inhibits the permeability of water that causes a major problem for germination. Seeds were analysed as recalcitrant and more than 90% of seeds were viable at the detached stage by the TTC test and decreased drastically to 30% after a year. During the period, considerable amount of fruits (Figure 5c and d) was infested with the larvae of flies; the seeds (2–3%) were also infested with *Fusarium* sp., *Lasiodiplodia* sp. and *Penicillium* sp. (Figure 5e). The fungi were borne as surface contaminants and were also present in the seed coat they penetrate deep into the seeds and attack the embryo, thus damaging the seeds to varying extents. Since the mother plant was found near the stream, most of the seeds were washed away to the plains during monsoon. Germinated seedlings also had slow growth rate and were highly prone to infection, which accounts for the absence of young seedlings in the natural habitat (Figure 5f). No further individuals were observed in the forest areas.

Freshly dispersed seeds were collected and analysed periodically for germination. Twenty-three seedlings were grown in the mist house in the host institute during the period of study. Seedlings thus developed from the viable seeds were panted nearby the mother tree. Continuous monitoring is going on to protect the species.

In recent years, studies on the reproductive biology of endangered tree species have gained importance throughout the world in order to make proper management and conservation strategies. Detailed studies on the reproductive biology of *E. blascoi* show that the tree performs a successive and higher fruit set percentage where seed germination is the limiting factor in the survival of the individuals and for successful seedling establishment in its own habitat. It requires alternative scientific methodologies to increase the seed germination rate. On the other hand, the extension of agricultural land in the forest area, monoculture of exotic trees such *Eucalyptus* sp. and *Acacia* sp. are the major factors for the fast decline in the population of *E. blascoi* due to natural habitat degradation.

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