MOEF PROJECT NO. 22/6/2010-RE, dt. 28.10.2010
Principal Investigator: Prof. A.J. Solomon Raju
ALL INDIA COORDINATED RESEARCH PROJECT

REPRODUCTIVE BIOLOGY OF FOUR RARE ENDANGERED AND THREATENED (RET) TREE SPECIES NAMELY, HILDEGARDIA POPULIFOLIA (ROXB.) SCHOTT. & ENDL., ERIOLAENA LUSHINGTONII DUNN (STERCULIACEAE), SYZYGIUM ALTERNIFOLIUM (WT.) WALP. (MYRTACEAE) AND SHOREA ROXBURGHII (DIPTEROCARPACEAE) OF ANDHRA PRADESH

Executive Summary of the Project

Reproductive ecology of four tropical tree species, namely, *Hildegardia populifolia* (Roxb.) Schott. & Endl. (lat. 14°06'N and long. 78°09'E), *Eriolaena lushingtonii* Dunn. (Sterculiaceae) (long. 16°02'N, lat. 78°57'E), *Syzygium alternifolium* (Wight) Walp. (Myrtaceae) (lat. 13°42N and long. 79°20E) and *Shorea roxburghii* Don. (Dipterocarpaceae) (lat. 13°40'N and long. 79°19E) were investigated in the Eastern Ghats of Andhra Pradesh State, India. Of these, *H. populifolia* is treated as an endangered species, *E. lushingtonii* as a vulnerable species while the other two tree species as globally endangered species. *S. alternifolium* is also an endemic species while *S. roxburghii* is also a keystone species.

Hildegardia populifolia is a deciduous tree species. All phenological events fruit dispersal, leaf shedding, flowering, leaf flushing and fruiting occur, one after the other during dry season. The flowering lasts 7-8 weeks at population level and 20-36 days at tree level. It is morphologically andromonoecious due to occurrence of male and bisexual flowers but functionally monoecious due to abortion of male function in bisexual flowers. It produces strikingly male-biased male and bisexual flower ratio; it is self-incompatible and obligately outcrossing. The flowers are nectariferous and the nectar has hexose-rich sugars, some essential and non-essential amino acids. *Trigona* bee and *Rhynchium* wasp were the exclusive foragers; their foraging activity does not promote cross-pollination. Male flower number, the pollen output, pollen characteristics and placement of anthers on the top of androphore conform to anemophily. The natural fruit set does not exceed 5%. The fruit is 5-follicled with 1 or 2 seeds. The low fruit set is compensated by the production of more 2-seeded follicles. The fruit characteristics such as wing-like follicles, membranous follicle sheath and very light weight characterize anemochory. Seeds with hard coat do not germinate readily during rainy season and their germination depends on the soil chemical and nutrient environment. The soil is deficient in nitrogen, potassium and phosphorous.

Eriolaena lushingtonii is distributed in dry and moist deciduous areas in the southern Eastern Ghats of Andhra Pradesh. Leaf flushing occurs during late dry season and flowering during early wet season. Fruit maturity and seed dispersal events occur during February-March. The flowers are either solitary or few-flowered cymes, borne in axillary position and do not stand out very prominently against the foliage and usually go unnoticed by the flower visitors due to the flowering pattern characterized by the production of a few flowers per day at tree level and emergence of herbaceous flora and leaf flushing in deciduous tree species following rainfall in June. The floral characteristics such as morning anthesis, exposed dehisced anthers presenting pollen and exposed flower base presenting nectar after anthesis indicates that they are adapted for day-active flower foragers for pollination. Certain co-occurring and co-flowering nectariferous and polleniferous herbaceous plant species attract a variety of insects in the habitat of *E. lushingtonii* and in effect the latter species receives reduced rates of foraging visits which contribute to both self- and cross-pollination.

In *E. lushingtonii* with hermaphroditic sexual system and weak protandry facilitates both self- and cross-pollination. The ability to fruit through both modes of pollination is adaptive but both are essentially insect-dependent since the extension of the stigmatose style beyond the height of stamens and the sticky nature of pollen grains which is further dampened by the high humidity during wet season preclude the occurrence of autogamy. The fruit set rate is low but it is partly compensated by the production of several seeds in *E. lushingtonii*. Seed dispersal is characteristically anemochorous. Since the dry season with high winds contribute to top soil erosion in the absence of all herbaceous flora on the slopes, the germinated seeds in the early rainy season struggle to establish and in effect, a few eventually establish and grow slowly. Therefore, the rocky and nutrient-poor soils, the pollinator limitation, bud and anther predation, seedling establishment problems and local uses collectively contribute to the endemic and endangered status of *E. lushingtonii* in the Eastern Ghats.

S. alternifolium is not an annual flowerer. The flowering intensity varies during the years of flowering. The flowering is either massive or sparse; in case of sparse flowering, the flowering is confined to a few branches

only. The floral traits suggest a mixed pollination syndrome involving entomophily and anemophily together called ambophily. Further, the floral traits suggest generalist pollination system adapted for a guild of pollinating insects. The plant is self-incompatible and obligate out-crosser. The flowers are many-ovuled but only a single ovule forms seed and hence fruit set and seed set rates are the same. Natural fruit set stands at 11%. Bud infestation by a moth, flower predation by the beetle, *Popillia impressipyga* and bud and flower mounds significantly limit fruit set rate. The ability of the plant to repopulate itself is limited by the collection of fruits by locals due to their edible nature, short viability of seeds, high seedling mortality due to water stress, nutrient deficiency, erratic rainfall or interval of drought within the rainy season. Therefore, *S. alternifolium* is struggling to populate itself under various intrinsic and extrinsic factors.

In *S. roxburghii,* massive blooming, drooping inflorescence with pendulous flowers, ample pollen production, gradual pollen release as a function of anther appendage and aerodynamic pollen grains– all suggest anemophily. The characteristics of nectar secretion, hexose-rich sugars and amino acids in nectar are additional adaptations for entomophily. The plant is therefore both anemophilous and entomophilous; the existence of these two modes of pollination is referred to as "ambophily". Since the plant is adapted also for entomophily, it attracts different insect species, bee, wasps, flies and butterflies. The nectar is a source of some of the essential and non-essential amino acids for insects. The ability to have both anemophily and entomophily is adaptive for *S. roxburghii* to set fruit to permitted level through cross-pollination. The natural fruit set does not exceed 15% despite the plant being ambophilous. Scarabaeid beetle by causing flower damage and bruchid beetle by using buds, flowers and fruits for breeding greatly affect fruit set rate and hence the success of sexual reproduction in this plant species. Anemochory is the mode of fruit dispersal but it is not very effective due to semi-closed nature of the canopy cover of the forest by the time the fruits mature. Seeds are recalcitrant, non-dormant, embryo is chlorophyllous while the fruits are on the plant. Healthy seeds germinate as soon as they reach the forest floor but their establishment is seemingly affected by the resource constraint due to rocky habitat. The study suggests that massive flowering for a short period, high bud/flower and fruit infestation rate, absence of seed dormancy and rocky habitat with nutrient-deficient soil disallowing seedling establishment collectively appear to be contributing to the endangered status of *S. roxburghii.*

In summary, all the four studied tree species play an important role in supporting insect fauna in deciduous forest ecosystem in the Eastern Ghats. *E. lushingtonii, S. alternifolium* and *S. roxburghii* are morphologically and functionally hermaphroditic while *H. populifolia* is morphologically andromonoecious but functionally monoecious. *E. lushingtonii* is primarily out-crossers while the other three species are self-incompatible and obligately outcrossing. *S. alternifolium* and *S. roxburghii* are ambophilous; the former is zoochorous while the latter is anemochorous. *H. populifalia* is primarily anemophilous while *E. lushingtonii* is entomophilous; both the species are obligately anemochorous. All the four tree species are nectariferous and offer pollen and nectar as floral rewards to pollinators.

The floral rewards in all the four tree species attract one or more than one class of insects - bees, wasps, flies, thrips and butterflies. The study clearly suggests that entomophily is effective, reliable and economical in terms of resource investment in the production of flowers and floral rewards. Anemophily functional in *H. populifolia* and *S. roxburghii* are effective. In *S. alternifolium,* both entomophily and anemophily function effectively. The buds and flowers of *E. hookeriana* and only flowers of *E. lushingtonii* are subjected to infestation by a beetle (not identified); the beetle uses the buds or flowers for feeding purpose only. In *S. alternifolium,* the buds are subjected to infestation by a moth (not identified); the moth uses the buds for breeding purpose only. The beetle in case of *E. lushingtonii* and moth in case of *S. alternifolium* have been considered to be natural controls to regulate the fruiting.

The study suggests that interactions between insect pollinators and the tropical tree species are important for the structural and functional integrity of the deciduous forest ecosystem. Further, flower and fruit predation and infestation by beetles and moth partly contribute to the endemic and endangered status of the studied tree species. Seed collection from natural areas and raising seedlings in experimental plots and nurseries for subsequent transplantation into natural areas seem to be imperative for expanding the population size of each of the studied tree species in the deciduous forest ecosystem with feasible soil nutrient amendments. The work reported here would form the most important information in order to take up further studies for effective conservation and management measures for the sustainability and restoration of population size of the studied tree species.

EXECUTIVE SUMMARY OF THE PROJECT
"Reproductive Biology of Five RET Species of Central and Western Himalaya"

Sanctioned to Prof. A. K. Bhatnagar (No. 22/2/2010-RE)

The work was carried out on our RET species of Central and Western Himalaya namely *Pittosporum eriocarpum*, *Engelhardia spicata*, *Acer oblongum*, *Acer caesium* and *Ulmus wallichiana*. The major finding of the research work are presented below:

Populations of ***E. spicata*** comprise of staminate, pistillate and andromonoecious trees. Staminate trees bear only male flowers and pistillate trees bear only female flowers. Monoecious trees bears all the three types of flowers i.e. male, female, and bisexual flowers which are functionally male. Monoecious trees show reciprocal heterodichogamy with a proportion of trees showing protandry and other proportion showing protogyny. No sexual lability was observed in the gender expression of monoecious trees. Staminate trees bloom earlier than the pistillate and monoecious trees. However, the flowering is synchronized amongst sites. Trees of E. spicata are complete out-crossers and are wind-pollinated. Fruit set through open-pollination in pistillate trees is 28.46-30.35 % and in monoecious tree is 45.60-52.31 %. However, geitonogamy yields very low (9.96±3.34) amount of fruit set. Thus, the species is mainly self-incompatible. Seeds are small, light-weight and winged. Seed dispersal occurs through wind and propagules were located up to 110 meters away from the source plant. Seeds require shady and moist places for germination, and thus high density of seeds was found germinated under the parent canopy. However, the seedling survival rate was almost negligible. Habitat fragmentation is having highly negative impact on the per cent fruit set and vigour of the seeds and seedlings. Regeneration in terms of seed germination and seedling survival is affected due to low viability of seeds, low germination and reduced survival of seedlings owing to lantana invasion and anthropogenic disturbances such as construction and farming in the under canopy areas. The tree is locally exploited for fire-wood, fodder and manuring.

Populations of ***P. eriocarpum*** comprise of staminate and pistillate trees. The flowers produced in staminate trees have cryptically functional pistillode. Gender ratios across all the sites were male biased. Trees show lability in gender expression with prominence of male to female transitions. Trees are complete out-crossers and are majorly pollinated by different pollinators at different study sites. A significant variation in the pollinator guild was also observed across study sites which was constant across seasons. The trees are mostly pollinated by bees in Uttarakhand and 77 by butterflies in Himachal Pradesh. The fruit set through open-pollination was 35.95 % and 56.42 % at Nainital and Kullu, respectively. Additionally, the seed set per fruit at both the sites was found to be pollen-limited due to the absence of major pollinator groups However, no instances of apomixis or fruit set through wind were noted at any of the sites. The seed and fruit dispersal occur primarily through gravity and surface run-off and to a lesser extent through ornithochory (endozoochory) at all the study sites. Seeds dispersed through gravity are mostly (79.52 %) deposited under the canopy. However, the seeds dispersed through birds were found up to a distance of 3 kms. The dispersal was limited across all habitats due to the absence of the disperser agents in most of the diurnal hours. Under natural conditions, the seed germination requires scarification treatment in the birds gut for germination. Thus, both the seed germination and seedling recruitment are limiting factors for regeneration of the plant. Additionally, no instances of dispersal through nocturnal agents were recorded. A major barrier in regeneration of

the plant is the underlying morpho-physiological dormancy of the seed which requires a time-period of 7-8 months for overcoming dormancy under natural conditions. However, this time-period was successfully reduced to two weeks using gibberellic acid and temperature stratification treatments. Density of the trees is a factor in attracting pollinators as well as disperser agents was found to be crucial as fruit set owing to the contribution of the pollinators and dispersal through bird species was found to be significantly correlated with the density of flowering and fruiting trees. Anthropogenic disturbances in the form of habitat fragmentation, lime quarrying, uprooting and pollarding of the plants for making highways, and usage of the plants as fodder in dry months are posing a threat to the species survival. Additionally, the tree is locally also exploited for fire-wood and manuring.

Acer oblongum is an andromonoecious tree species due to the presence of male and bisexual flowers but exhibits cryptic monoecy since each type is functionally either male or female. Tree bears three types of flowering morphs: Male Type I, hermaphrodite and Male Type II. Male Type I flowers bloom early in March during the beginning of the flowering season and are meant primarily to attract pollinators and sustain them (mainly bees) till the blooming of hermaphrodite and Male Type II 78 flowers. However, the phenology of shade oriented trees of A. oblongum varied with those growing in sun. Flowers of A. oblongum promote outcrossing since the bisexual flowers which set fruit have indehiscent anthers. Pollen from Male Type II flowers pollinates the co-flowering hermaphrodite flowers. So, mode of pollination is facultative xenogamous. A. oblongum shows mixed syndromes of anemophily in pollen characteristics and entomophily in floral structure. In A. oblongum natural fruit set is found to be 33-40%. However, this is primarily due to pollen limitation and high pollen sterility. Also wing loading in seeds of A. oblongun sometimes results in formation of hollow fruits. Mature fruits do not invariably indicate presence of mature seed inside. In A. oblongum seed germination and seedling establishment do not pose any problem since seeds do not require any pre-treatment but insect infestation of seeds was observed in few trees. This species prefers growing in slightly acidic to neutral soil (pH ranges from 6.0-7.0) and grows well in moist soil. The tree is commercially exploited for its valuable timber and leaves are used for manuring.

Acer caesium is dioecious, with distinct male and female trees in the population. The natural populations show male-biased sex ratio i.e. presence of larger number of male trees than female trees in a population. The presence of focal female tree ensures the larger number of pollen donor male individuals in a population which effects the seed set and regeneration. The inflorescence of *Acer caesium* is cymosely branched terminal corymb. Floral structure promotes wind pollination as the presence of sticky papillate stigma is well exposed to wind and the male flowers have exerted stamens. The pollen grains have all characteristics of wind pollination. The mode of pollination is majorly by wind but insect visitors also contribute to pollination. Pollen limitation is observed within populations as pollen supplementation experiments resulted in higher fruit and seed set. The fruit set is very high as a result of successful pollination process but mortality of flowers at different post-pollination developmental stages reduces the successful seed set percentage. At maturity, the pericarp colour turns dark brown. The change in fruit colour corresponds to a change in seed colour. The outer structure of the *A. caesium* samara attains full size within two months after anthesis, but its inner cavity requires an additional five months for seed fill. Mature fruits started dispersing through wind as a diaspore from late October to December. 79 Maximum distance travelled by the diaspores was 130 meters by wind. In its natural habitat, the samara is dispersed in late autumn and undergoes stratification when it is

covered with snow. Physiological dormancy of different levels is found in seeds from different populations. Seed germination requires prolonged chilling and pricking of pericarp and testa in natural conditions. Seedling establishment rate is very low at forest floor as some of the seeds are not mature and get degraded by pest infestation under seed bank and many of them lose their viability. Successful regeneration needs artificial germination and seedling establishment in the nurseries and introducing the sapling to forest floor after hardening stage.

Flowers of ***Ulmus wallichiana*** are hermaphrodite arranged in lateral racemes. The anthesis time largely varies due to morning temperature. The flower structure promotes wind pollination. Insect visitation is insignificant and wind pollination is responsible for maximum fruit-set. Breeding experiments reveal that seed set is higher in manual pollination. Effective pollination time is 0700 to 1200 hrs. Fruit set is very high but the number of infertile or hollow fruits is very high which is due to high rate of abortion and mortality during seed maturation. U. wallichiana diaspore is flat, obovate and with membranous reticulate wing completely surrounding the seed. The winged diaspore is primarily dispersed by wind. The maximum dispersal of seeds is 180 meters. Seeds are orthodox and lose viability and germinability within a few months under natural conditions after dispersal. Seeds can be stored under low temperature and specific moisture content for long artificially. Regeneration rate is very low in forest floor. Seedlings survival rate is relatively high at higher altitude populations. Deliverables: The investigated tree species belong to RET category. The reason behind their scarce distribution and lack of regeneration in their natural habitats needs to be understood. Reproductive data indicates that the investigated species show low numbers due to pollinator constraints and low rate of fruit/ seed set. The rate of seed abortion is high. Seed germination rate is also very low indicating inbreeding depression.

Executive Summary

All India Coordinated Project on Reproductive Biology of RET tree species

PI: Dr. Rajesh Tandon, Professor, Department of Botany, University of Delhi, Delhi – 110007.

Title: Reproductive Biology of Rare, Endangered and Threatened (RET) Tree Species namely: *Anogeissus sericea*, *Salvadora oleoides*, *Tecomella undulata* and *Wrightia tomentosa* in Rajasthan.

The selected tree species have been worked out for the first time. The base-line information would be useful in conservation of the tree species. The information would be of immense importance for government agencies heading the forestry and agroforestry departments and the agri-farm owners. Specifically the information generated on the reproductive strategies of the tree species would help in (i) species reintroduction, and (ii) designing their conservation at the community level, as their survival is highly dependent on ecosystem service provided by the legitimate pollinators. Importantly, three out of the four tree species exhibit specialized pollination syndrome. During the preliminary survey it was noticed that there were two varieties of *Anogeissus sericea viz.* var. *sericea* and var. *nummularia.* Therefore, both the varieties were included in the investigation. Both the taxa were self-compatible; excessive autogamy in the variety *nummularia* incurs considerable amount of inbreeding depression. However, var. *sericea* remained unaffected by inbreeding depression. Pollinator limitation was more prominent in the var. *sericea* than in var. *nummularia. Salvadora oleoides* is a self-compatible tree species. Although a large number of fruits are produced in nature, natural seedling establishment was negligible. Some of the main factors for poor regeneration this species are inbreeding depression, poor seed viability and germination. In Rajasthan, *Wrightia tomentosa* is represented by only one population, where only a few individuals produce flowers. Thus, ineffective population size of mature trees may be one of the factors for poor natural regeneration in the species in addition to overexploitation for its white textured wood. This may be due to inbreeding depression and low seed viability. 'Roheda' or *Tecomella undulata* is an obligate self-incompatible and ornithophilous. The flowers are frequently robbed of their nectar by nectar robber birds. Intense nectar robbing influences the pollinators to visit additional flowers and trees thereby increasing the female fitness. The natural recruitment of this threatened species requires optimal nutrients in the initial stages of establishment. For this, the practice to grow them as

agroforestry tree species on the seasonally cultivated plots would not only continue to profit the farmers but would also safeguard its conservation in moisture limited environment.

Key recommendations based on the work

The present work demonstrates that heterogeneity at the population level is crucial for the reproductive success in *Tecomella undulata*. The other three tree species are self-compatible but definitely not autonomous. Thus fruit set is facilitated by the essential requirement of pollinators. This information underlines the importance of species organization in ecosystem context. For establishing new populations, the unrelated young plants/genets should be introduced from different regions. Monoculture of a tree would be ineffective as the pollinators presence in the community should persist in the community through continued availability of resources. Any effort to reintroduce the species in new habitats should consider interplay of plant-pollinator interaction network at the community level for the sustenance of the tree species investigated. Tree density is a crucial issue at least in the varieties of *Anogeissus* as they are pollinated by dipteran flies which are slow foragers and their activity is highly restricted. Also, to avoid the neighbourhood effect that often leads to inbreeding depression, reintroduction of unrelated sibs is crucial. The site at *Kewde ki naal* in Udaipur needs to be protected from grazing pressure as the flowering individuals are very few in number. To circumvent the effect of founder effect, the other age-groups of the tree must be guarded to become reproductively active. The survival of trees needs to be monitored at the site.