**Abstract**

Pollen dispersion and frequency of flowering were studied in the natural population of *Pinus roxburghii* Sargent (Chir pine) at altitudes between 1600 and 1900 m above sea level in the Tehri forest division of Garhwal Himalaya. Temporal variations for pollen cone and male strobilus production were observed with a year of bumper production. An individual tree produced $14.96 \times 10^{11}$ to $2.64 \times 10^{11}$ pollen grains with a range of variation in the population between $12.95 \times 10^{11}$ and $19.09 \times 10^{11}$ pollen grains. Dispersion of the pollen grains from the fringe of the forest declined sharply with distance. The results implied that a segregation barrier of 640 m would be the minimal distance for the management of a Chir-pine seed orchard at the study location.

**Keywords:** Chir-pine, pollen cone, gene flow, Himalaya

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**1. Introduction**

*Pinus roxburghii* Sargent, commonly known as Chir-pine or Himalayan Long needle pine, covers a wide range of distribution in the Himalaya as pure forests between 450-2300 m above sea level. The species also occurs in association with other coniferous and broad-leaved species, such as *Cedrus deodara* (Roxb.) G.Don, *Lyonia ovalifolia* (Wall.) Drude, *Myrica esculenta* Buch.-Ham. ex D. Don, *Pinus wallichiana* A. B. Jacks, *Quercus leucotrichophora* A. Camus ex Bahadur, and *Rhododendron arboreum* Sm. towards its upper limit and *Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Bedd., *Bauhinia variegata* L., *Ougeinia oojjinenses* (Roxb.) Hochr., and *Shorea robusta* Gaertn. towards the lower limit. At upper elevations it favors warmer slopes and drier spurs. Several characteristics such as high regeneration potential (Kumar and Bhatt, 1990), ability to inhabit on degraded habitats (Joshi, 1990), fast growth (Misra and Lal, 1984), straight cylindrical bole and high volume returns (Singh, 1979) and the capability to yield a substantial quantity of resin (Deshmukh, 1966) make this species a valuable reserve in Himalayan region. It has immense significance in the Indian economy, since the resin it yields has various uses in a variety of industries.

The conversion from the vegetative to the reproductive stage is one of the important morphogenetic events in the lifespan of a long lived tree species. It carries multiple changes in the metabolism, and stimulates numerous decisive processes linked with the commencement of sexual reproduction and alternation of generation (Chailakhyan, 1968). During alteration from the vegetative to the reproductive phase, the cells accountable for the development of sex organs attain extremely diverse biochemical capacities compared to the vegetative growth of the apical meristems (Chailakhyan, 1979; Zeevaart, 1976). The result is the formation of reproductive buds and the initiation of reproductive structures of the different sexes. Further, the male and female plants/flowers also differ from each other in possessing a few specific macromolecules, which constitute the molecular markers or probes of differentiation (Jaiswal & Kumar, 1983).

Pines are anemophilous and monoecious, that is the male and female reproductive structures are borne on the same tree but are separated in space. Wind-borne pollen offers homogeneity among populations and ensures that most patterns of geographic variation will be clinal (Ledig, 1998). The result is that each tree is expected to be pollinated largely
by its neighbor. For example, Colwell (1951) calculated that more than 90% of the pollinations on a known tree would come from the adjoining trees on its windward side. Contrary to this, Koski (1970) found that a single Pinus sylvestris L. tree would account about 10% of the pollen received by a close neighbor. Although half of the pollen received by a given tree in P. sylvestris stands is derived from trees within a 50 m radius, that area would include at least 25-30 trees (Koski, 1970). Thus, the gene flow, through pollen exchange in Pinus, is so great that recombination is maximized within a population and random genetic drift which leads to segregation among populations is retarded. High levels of recombination produce the required variation to adjust to altering conditions. Dispersion of pollen is an inactive process which depends on factors such as pollen production values of a tree, sunshine, wind, humidity, and rain. Pollen production varies from tree to tree, location to location, year to year, species to species, and age of the tree. Gene movements in forest trees entail both pollen and seeds (Smouse & Sork, 2004), which is responsible for genetic connectivity among individuals in populations and established genetic diversity in the population (Sork & Smouse, 2006). Pollen dispersion is the movement of the male gamete and its pattern is significantly influenced by the genetic structure and effective size of the plant populations (Sousa & Hattener, 2003). Additionally, the extent of pollen immigration also determines the rate of contamination in an isolated population. Pollen gene flow is one of the most important factors that influences genetic structure of wind pollinated forest trees (Burczyk et al., 2004). Wind borne pollen of broadly distributed tree species has the potential to travel dozens or hundreds of kilometers (Di-Giovanni et al., 1996; Lindgren et al., 1995; Rogers & Levetin, 1998). However, sterile or unviable pollen, which are light in weight, can travel long distances (<2 km) (Erdman, 1943). Such distant dispersal of pollen is not of much interest in pollination ecology, but the area of huge pollen deposition from the source is of great interest (Faegri & van der Pijl, 1979). Therefore, it is important to know the successful long distance dispersal of pollen grains or what is the distribution of effective pollen distances. An attempt has been made in this research to discover the effective pollen distribution distances and pattern and level of pollen output per tree in P. roxburghii during four consecutive years.

2. Materials and Methods

This study was conducted during four consecutive years in January, February, and March, 2013, 2014, 2015, and 2016, in the Gair-pine forests of Ranichauri, Tehri Forest division at the altitude range of 1600 and 1900 m above sea level (Latitude 30° 18’ N, Longitude 78° 24” E) (Figure 1). The study location is situated in the central part of Western Himalaya. The mean annual temperature and total annual rainfall at the study location are 14.2±2.06 °C and 1785.00 mm, respectively.

2.1 Assessment of reproductive phenology

In order to monitor the development of pollen and seed cones, long shoots containing pollen and seed cone buds were randomly sampled from 25 different trees at weekly intervals from mid-January to Mid-February and two times per week between mid-February and March, until pollination ended. To observe pollen development, individual microsporangia were dissected from the fresh pollen cones of several trees. Individual microsporangia were squashed in acetocarmine on microscope slides, cover slips were applied, and the slides were heated to intensify staining (Johansen, 1940). The pollen grains were assessed using binocular microscope.

2.2 Assessment of pollen cones and pollen production

Twenty-five candidate trees from the natural forest were selected and marked by yellow paint. These trees were considered as representative individuals for every year of sampling. Height, diameter, and maturity were used to select the trees based on the check tree method or comparison tree method. The selected candidate trees represented the entire study area of about 10 hectares. In order to estimate the production traits in each candidate tree, the main branches were counted, a sample of ten branches were selected randomly, and then all of the pollen cones were counted. Furthermore, 50 pollen cones that were scattered throughout the trees were harvested and the numbers of pollen strobili per pollen cone were counted for each tree. Apart from each pollen cone, pollen strobili were chosen from the lower, middle, and upper parts and the numbers of microsporangia were counted manually. Assessments of the pollen grains were done on five microsporangia from different pollen strobili of each tree which had been previously measured. Basically, the method used was that of Molina et al. (1996). In order to estimate the total production of pollen grains per tree, the total number of microsporangia per tree were calculated by multiplying the total number of pollen cones by the average number of pollen strobili per pollen cone, and then by the average number of microsporangia per pollen strobili. The result was then multiplied by the average number of pollen grains produced per microsporangium.

2.3 Statistical analysis

The effects of years and altitudes on the number of pollen cones and pollen strobili per tree, microsporangia, and pollen grains per tree were analyzed by means of split-plot
analysis of variance (ANOVA) with nesting. Years and altitudes were examined as fixed effects. Counts were log transformed in order to improve normality of residuals and to reduce heteroscedasticity (Sokal & Rohlf, 1995). ANOVA was performed using SPSS version 14.

2.4 Observations on the pollen dispersion

Pollen dispersion through the air was recorded using jelly coated microscopic slides. Ordinary microscopic slides covered with a thin coat of petroleum jelly or Vaseline, were used as pollen traps. The slides were mounted vertically on wooden rods that were 1.5 m in height and placed around the source trees at geometrically increasing distances: 0, 0.5, 10, 20, 40, 80, 160, 320, and 640 m. The pollen traps were unprotected and exposed to the air throughout the period of observations. The pollen traps were set out between 9:00 and 10:00 am from the fringe of the forest in two possible directions (horizontal and uphill direction) and collected after 24 h. The pollen count was made directly from the exposed glass slides under a binocular microscope and the area counted per slide was fixed at 1 cm² (1×1 cm). The experiment was repeated in open conditions to assess the efficiency of pollen flow inside the forest.

2.5 Mathematical treatment of pollen dispersion data

Pollen dispersion distances are best presented in terms of a curve. Bateman (1947) found that the function F = Fo.e^kD fits the dispersion data very well (Equation 1). In this equation, F and Fo are pollen frequencies at distances D and 0, respectively, and e is the base of the natural logarithms. If the curve is reduced to a straight line, the quantity k is the slope.

\[ F = f(D) = Fo.e^{kD} \]  

The logarithmic transformation of log₁₀e is:

\[ \log F = \log Fo - (\log₁₀e)kD = \log Fo - 0.4343kD \]

3. Results

The first step in sexual reproduction is the initiation of floral buds. In Pinus roxburghii pollen cone bud initiation was recorded in the last week of January with 26-33 days required for complete maturation. However, the initiation of ovulate strobili was recorded in the first week of March which reached full receptivity within one week and remained receptive for up to 5 days.

The number of pollen cones, pollen strobili, microsporangia, and pollen grains per microsporophyll oscillated considerably among trees in the population and varied considerably from one year to the next. The number of branches that produced pollen cones per tree in the years 2013, 2014, and 2016 varied from 27 to 48. The diameter and height of the selected trees oscillated between 71.00 cm and 86.00 cm and 34 m and 41 m, respectively. The number of pollen cones per terminal branch and pollen strobili per pollen cone varied from 498±21.52 (year 2015) to 734.0±114.37 (year 2014) and 79.4±14.24 (year 2015) to 121.15±15.12 (year 2014), respectively. Among the four years, 2014 was recorded as the highest production year compared to 2013, 2015, and 2016. The number of microsporangia per pollen strobili oscillated from 109.2±6.47 to 129.13±14.14. Similarly, the number of pollen cones and pollen strobili per tree in the years 2015 and 2014 varied from 19.18×10³ to 35.30×10³ and 2489.58×10³ to 3278.56×10³, respectively. The average number of micro-sporangia per tree in 2015 and 2014 changed from 325637.22x10³ to 38365,9x×10³ and the production of pollen grains per microsporangium changed from 5269,72x10³ to 6901±122.17. Ultimately the number of pollen grains per tree ranged between 1295.9x×10³ in the year 2015 and 1908.51x10³ in the year 2014.

In P. roxburghii, the number of pollen cones, pollen strobili, microsporangia, and pollen grains per tree varied considerably during the years and within the population. An analysis of pollen production per tree revealed significant interactions between the years (F=44.45, P=0.0014), individuals (F=68.24, P=0.0001), and year × individuals (F=29.94, P=0.0076). There were also significant differences among the years, individuals, and year × individuals interactions in the number of pollen cones (F=16.25, P=0.0025, F=28.46, P=0.0001, and F=9.78, P=0.0056, respectively), pollen strobili (F=19.56, P=0.0034, F=37.62, P=0.0001, and F=14.37, P=0.0047, respectively), and microsporangia (F=32.25, P=0.0024, F=54.74, P=0.0001, and F=19.62, P=0.0062, respectively) per tree.

The data on pollen migration from the source revealed that the pollen frequencies were highest in all directions near the source trees. The distribution of the majority of pollen grains from the source was observed up to 40 m towards uphill and horizontal directions. The frequencies of pollen grains to the source frequency at these distances were 28.83 and 23.34, respectively. The results also revealed that the pollen grains could travel up to 320 m and 640 m in the uphill and horizontal directions, respectively (Figure 2). The averages of pollen to source frequency at these distances were 2.35% and 2.16%, respectively.

4. Discussion

Flower formation starts when a vegetative meristem suddenly changes its pattern of division to produce beginnings of floral organs and appendages. This change is influenced by a number of factors; however, specifics are lacking on the internal control, which causes one meristem to produce a flower and another to remain vegetative (Schopmeyer, 1974).

![Figure 2: Pollen dispersion from source tree in two directions.](image-url)
Climatic factors influence the anthesis, androecia protrusion and anther dehiscence, and hence pollen release. In *P. roxburghii*, air temperature must exceed (20 °C) and relative air humidity must remain below 85% to begin the process of anthesis. However, high relative air humidity favors androecial protrusion. Another dehiscence and pollen release are also affected by relative air humidity. Anthesis also showed a diurnal periodicity every day from 0600 to 1800 h; however, anthesis was not affected by the presence or absence of light (Khanduri and Sharma, 2000).

An analysis of pollen production clearly indicated temporal and tree to tree variation in pollen production in *P. roxburghii*, which ultimately suggested that climatic conditions along with genetic makeup of individual tree played a significant role in this variation. A bumper pollen yield was observed in the year 2014.

In forest trees, determination of the extent of pollen migration is important because trees are sessile and the gene flow of interest is valuable to know the background of external pollen sources into the seed orchards. Gene flow from unselected, background pollen sources into a seed orchard results in pollen contamination, which affects the genetic quality of seed yield. The intensity of pollen contamination varies with the species, mating pattern of the species, synchrony in reproductive phenology of the trees of within and outside-seed orchard, size of seed orchard and its background population, extent of isolation, and the environmental conditions. A number of studies have reported pollen contamination for several species with the rate of contamination that varied from almost 0 (El-Kassaby & Ritland, 1986) to 90% (Fast et al., 1986; Kaya et al., 2006). The rate of contamination may vary from one year to the next, for example in Douglas-fir seed orchard the contamination rate in one year was 44% and in the next year it was 89% (Fast et al., 1986). Contrary to this, a regular level of contamination between 69 and 71% over a period of three years was recorded in a Norway spruce seed orchard (Fast et al., 1986). Contamination affects genetic gain, genetic quality, and genetic diversity in different ways. As pollen contamination increases, the genetic gain of the seed crop decreases (Wheeler & Jech, 1986) and genetic diversity increases up to a certain level (Lindgren & Mullin, 1998). A high level of pollen contamination may either increase or decrease the genetic diversity of a seed crop that impacts the conspecific population and its genetic characteristics (Adams & Kunze, 1996). Under the condition of absolute pollen contamination (100%), half of all genes come from the parents within the orchard because the pollens are the male component of reproductive success (Funda & El-Kassaby, 2012).

The scattering pattern of pollen grains in the population implies successful seed set through mixed mating. The outcrossing rate in *Pinus* was reported to be high at the population level (Sharma et al., 2012). A decrease in pollen concentration with increased distance implies a reduction in percentage seed set with increased distance as noted in Douglas fir (Wright, 1953). The amount of pollen scattered over long distances is small in comparison to the magnitude of pollen formed by an individual. The distance that wind dispersed pollen travels has been the topic of several investigations and it was confirmed that a single pollen grain can be carried long distances; however, it is uncertain that it would be viable after such a long journey (Bateman, 1947).

Dispersal varies greatly due to factors such as the wind, height of vegetation, and length of period of release. Also, the area of pollen coverage can vary from a few meters to hundreds of meters, and under exceptional circumstances can be more than a kilometer (Raynor et al., 1969).

The results of this study revealed a large pollen pool in the population due to copious pollen production per tree which exhibited high levels of pollen dispersal for *Pinus roxburghii*. The estimates of average pollination distances varied between 320 and 640 m. This suggested that trees in *Pinus roxburghii* mate with a large number of conspecific neighbors distributed over a large area in their vicinity. The pollen dispersal distance estimates from this study appeared to be larger than other coniferous tree species, such as 91.4 m for *Pinus edulis* Engelm., 45.7 m for *Pseudotsuga menziesii* (Mirb.) Franco, 100.6 m for *Picea abies* (L.) H. Karst., 213.2 m for *Cedrus atlantica* (Endl.) Manetti ex Ca. (Wright, 1952), 152.4 m for *Pinus elliottii* Engelm. (Wang et al. 1960), and 190 m for *Cedrus deodara* (Roxb. ex D. Don) G. Don (Khanduri and Sharma, 2002). A segregation barrier of 600 m in *Pinus roxburghii* was also observed by the author at Pauri Garhwal District (Khanduri, 2012). The decline in the frequency of pollen deposition with increasing distance from a pollen source is an issue of utmost importance in the setting up and management of a seed orchard. For example, Wright’s (1952) data on *Pinus edulis* Engelm (Pinyon tree) and Wang et al. (1960) data on *Pinus elliottii* Engelm (Slash pine), showed a rapid decline in pollen deposition with increasing distances. The occurrence of pollen of Pinyon at 91.4 meter was 1% of the frequency at the pollen source, and in the case of Slash pine, it was 2-5% of the source frequency at 152.4 meter.

5. Conclusions

In *P. roxburghii*, the occurrence of pollen at 640 m was 2.16% of the source frequency, which implies that a segregation barrier of 640 m could be used for the establishment of a Chir pine seed orchard.

References


