Pollen cone characteristics, pollen yield and pollen-mediated gene flow in *Cedrus deodara*

Cedrus deodara (Deodar Cedar) is a highly important timber tree of western Himalaya, occurring naturally in pure stands or in association with other Himalyan conifers between the attitudinal range of 1500 and 2600 m asl (occasionally between 1200 and 3600 m asl). The tree looks pyramidal with pendulous branches at the young stage[1,2]. Generally, an individual tree attains a height range between 15 and 50 m at maturity with its drooping leader and the needle length varies between 2 and 6 cm. Deodar is a wind-pollinated monoecious species. The pollination occurs in autumn and the seed cones mature after 17–18 months[3,4]. Quantitative relationships between pollination efficiency and pollen availability for natural populations of this species have not been developed so far. Therefore, our aim in this study was to evaluate: (i) microsporangium dehiscence in relation to certain environmental factors and time of the day, (ii) variations in pollen output per strobilus and per tree, and (iii) spatial dispersion of pollen from isolated single tree.

Mating system has prime importance to theoreticians, field biologists and plant breeders, because it determines the distribution of genotypes within populations and influences the degree of differentiation among populations. Outcrossing promotes gene flow. Lower levels of gene flow permit higher levels of differentiation among populations. The importance of the mating system is reflected in the ongoing interest in its evolution[5] , the advent of paternity analysis[6], population structure[7] and gene flow[8].

Pollen gene flow has long been considered a major concern in tree improvement programmes. It is one of the most important factors influencing genetic structure of wind-pollinated forest trees[9]. Wind-borne pollen grains of widely distributed tree species have the potential to travel dozens or hundreds of kilometres[10,11]. But it is important to know the successful long-distance dispersal of pollen grains or the distribution of distances of the effective pollen. An attempt has been made here to know the effective pollen distribution distances in *C. deodara*.

The study was conducted during two successive flowering seasons, October 2000 and October 2001, in the deodar forests of Garhwal Himalaya, India at two different locations (Table 1).

To monitor the development of pollen cone and seed cone, long shoots containing pollen and seed cone buds were randomly sampled from five different trees at every two weeks interval between mid-June and mid-August; every week interval between mid-August and mid-September, and two times per week between mid-September and mid-October, 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 heatened to intensify staining[12], and the pollen grains were assessed using binocular microscope.

Observations of microsporangium dehiscence began at the time of anthesis by sampling 10 strobili of *C. deodara* (which had just begun to flower) on five different trees at each location. Samplings continued until all the microsporangia in a strobilus had dehisced. Each strobilus was examined after every 2 h to identify the patterns of anthesis and dehiscence. Observations were made using a hand lens (20×) by scoring and removing to avoid duplication. The prevailing air temperature and relative humidity were also recorded close to the strobili studied during each observation, using a thermohygrometer.

C. deodara has a large number of strobili arranged mostly on the main branches of the crown. First, the main branches were counted, and then a sample of five representative branches was selected at random and their strobili were counted. In total, 20 strobili were sampled from each tree, and the number of microsporophylls was counted manually. Microsporophylls were obtained from closed strobili, kept in 70% ethanol, washed in distilled water, measured and placed in test tubes. The microsporophylls were crushed using a glass rod and the pollen grains were suspended in 1 ml distilled water. From this concentrate, five 10 µl droplets were removed and the pollen grains were counted using a binocular microscope. Pollen grains were counted on five microsporophylls from different strobili of each tree. The method used for pollen productivity analysis was modified after Tormo Molina *et al.*[13]. To estimate the total production of pollen grains per tree, the total number of microsporophylls per tree was calculated by multiplying the total number of strobili by the average number of microsporophylls per strobilus. The result was then multiplied by the average number of pollen grains produced per microsporophyll.

The effect of years and populations on the number of strobili per branch, strobili per tree, pollen sacs per strobilus, pollen sacs per tree and pollen grains per tree was analysed by means of split-plot ANOVA with nesting. Years and populations were examined as fixed effects. Counts were log-transformed in order to improve normality of residuals and to reduce heteroscedasticity[14]. ANOVA was performed using the Super Anova statistical package[15].

A representative deodar tree, situated on the agricultural fields of Premnagar village (altitude 1200 m asl) was selected as the pollen source. It was 21 m tall with a crown diameter of 8.0 m, and was isolated from possible contamination from foreign pollen by more than 1.60 km. Pollen samples were collected at increasing distances from the source tree: (1) parallel to the average wind direction and up to 192 m (at distances 0, 3, 6, 12, 24, 48, 96 and 192 m) horizontally from the source tree, with an average slope of ±5°; (2) up to 96 m away from the source tree in an uphill direction (average slope 23°),

Table 1. Details of the study sites/locations

Location	Altitude (m asl)	Longitude	Latitude	Stand density (individuals/ha)
Ghimtoli	2300	78°15′	30°23′	436 ± 29.77
Teka	1900	78°46′	30°8′	195 ± 12.71

Table 2. Strobilus and pollen production in *Cedrus deodara* during two years (values are average ± standard error)

Locality/ year	Flowered branches/tree	Number of strobili/branch	Strobili/ tree	Microsporophylls/ strobilus	Microsporophylls/ tree ($\times 10^3$)	Pollen grains/ microsporophyll	Pollen grains/ tree ($\times 10^9$)
2000							
Teka	33 ± 4.5	90.8 ± 28.7	3385.8 ± 864.4	409.0 ± 42.6	1188.2 ± 412.6	9098.4 ± 502.8	11.90 ± 4.02
Ghimtoli	28 ± 5.06	96.8 ± 34.2	3082.8 ± 804.5	352.2 ± 64.2	1098.8 ± 140.2	10094.4 ± 1650.8	12.20 ± 4.76
2001*							
Teka	14 ± 4.72	78.8 ± 44.6	824.6 ± 142.8	426.2 ± 60.8	406.42 ± 102.2	11225.6 ± 2245.2	5.62 ± 2.6
Ghimtoli	10 ± 3.82	62.8 ± 30.6	714.0 ± 202.4	346.4 ± 44.6	580.4 ± 102.2	10320.2 ± 1005.6	6.20 ± 3.2

*Flowering was totally absent in about 50% trees.

and (3) up to 768 m from the source tree in a downhill direction (average slope 32°). Pollen frequencies could not be measured beyond these specified distances because they became negligible and also because geographical barriers were present in the form of forests of other species, particularly banj oak (*Quercus leucotrichophora*). Samples were collected for 4 days during 2000 and 2002 (because flowering was totally absent in 2001 in the selected isolated trees). Twenty-five pollen slides were fixed daily at geometrically increasing intervals in three possible directions during the peak flowering periods of both the years.

Ordinary microscopic slides covered with a thin coat of petroleum jelly were used as pollen traps. The slides were fixed in a horizontal position on a 2.5 m long pole. The slides were unprotected and exposed to the open air. New slides were mounted every day between 1600 and 1700 h, and were collected after 24 h. Slides were covered with a cover slip when they were collected to protect the samples. Pollen counts were made in the laboratory using a binocular microscope. The area in which grains were counted was fixed at 1 cm^2 per slide, so that the frequency near the source tree ranged from 200 to 500 grains per slide, which gave a base for the estimates of frequencies at more distant positions. Analysis of one complete set of slides taken from a series of transects around a single source tree gives the slope of one pollen dispersion curve. Replications were made by duplication of complete transects on other days and in another year.

Bateman[16] found that the function $F = F_0 e^{-kD}$ fitted dispersion data well. In the above formula F and F_0 are pollen frequencies at distances D and 0 respectively and e is the base of natural logarithms. If the curve is converted to a straight line, the quantity k is the slope.

The pollen strobili of *C. deodara* are erect catkins that attain a length up to 5 cm and diameter 1.5 cm, appear solitary erect on the small shoots, bears high number of microsporophylls at a range between 260 and 480 per strobilus. Pollen strobili initiated in mid June, become dormant in the fall, growth restarted in mid-August, and pollen development occurred in mid-September to early October. Pollen cones quickly enlarged at meiosis and were green and compactly arranged. As pollen cones mature, they turn yellow and enlarge up to 8 cm in length, gradually separating the microsporophylls and dehiscence begins to shed pollen. In natural condition the microsporangium dehiscence occurs diurnal between 0800 and 1800 h with the maximum frequency during 1200–1400 h at both sites. After dehiscence of microsporangia, a slight breeze dislodges the pollen grains to the surroundings and a small proportion of it lands on the ovulate strobili. The pollen cones in a branch become empty over 3 to 5 days, depending on the weather conditions. By the end of October, pollen cones turn brown and most of the pollen is shed. Some pollen cones remain on the shoot and mostly fall on the forest floor.

The ovulate strobili are erect, cone-like structures of 1–1.5 cm length and 0.7 cm diameter, arrested by needles at the base. These are borne erect solitary at the tips of the dwarf shoots, and are less abundant than the pollen cones. The initiation of ovulate strobili buds did not occur until early August. During late August, the buds of ovulate strobili appeared and enlarged by the end of September. As scales developed, two ovule primordia were initiated on the basal adaxial surface of the fertile scales. Some scales in the basal portion of the cone lacked ovules or formed only rudimentary ovules. As seed-cone buds developed they elongated and bract-scale margins were visible and allowed pollen grains to pass between them. The margins of the bracts reflexed and curled downward. These processes created enough spaces between bract-scale complexes, and pollen readily passed into the cone with air currents. This stage appeared to be the most open and receptive, and may last for 3–5 days depending on the air temperature and relative humidity. By the end of October, scales had thickened enough to nearly seal the spaces between them.

In *C. deodara*, the number of pollen strobili per branch, microsporophylls per strobilus, strobili per tree, microsporophylls per tree, pollen grains per microsporophyll, and pollen grains per tree varied considerably in 2000 and 2001 at both locations. The average number of branches which produced pollen strobili per tree was 33 and 28 in the year 2000 and 14 and 10 in the year 2001 at both sites Teka and Ghimtoli respectively. However, about 50% of the trees at both sites in the year 2001 did not produced pollen strobili. The average rate of pollen production per tree was 12.20×10^9 and 6.20×10^9 at Ghimtoli and 11.90×10^9 and 5.62×10^9 grains per tree at Teka in 2000 and 2001 respectively (Table 2).

Analysis of pollen production per tree revealed significant year and population effects, but non-significant year × population interactions (Table 3). There were significant differences among years and populations in the number of pollen strobili per branch, strobili per tree and microsporophylls per tree. These variables also show non-significant year × population interactions. The analysis of production of microsporophylls per strobilus and pollen grains per microsporophyll were non-significant in year but less significant in population effect (Table 3).

Dispersion of pollen grains in *C. deodara* was recorded during the peak flow-

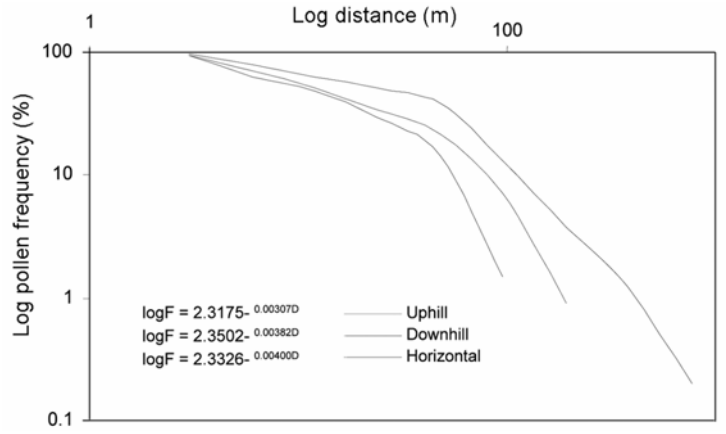

Figure 1. Pollen dispersion in *Cedrus deodara* from isolated single trees at three directions.

Table 3. ANOVA of the effect of year and population on the number of strobili, microsporophylls and pollen grains per tree in *C. deodara*

Response variable and source	d*f*	MS	*F*	*P*
Number of strobili per branch (R^2 = 0.784)				
Year	1	30.22	21.18	0.0001
Population	1	10.46	2.34	0.0014
Year × population	1	1.324	0.82	0.5216
Number of strobili per tree (R^2 = 0.598)				
Year	1	52.24	28.24	0.0001
Population	1	11.20	3.92	0.0012
Year × population	1	5.26	1.10	0.4252
Number of microsporophylls per strobilus (R^2 = 0.584)				
Year	1	34.12	18.16	0.2386
Population	1	14.42	10.22	0.0100
Year × population	1	5.14	2.30	0.2456
Number of microsporophylls per tree (R^2 = 0.770)				
Year	1	62.48	34.10	<0.0001
Population	1	30.28	20.16	0.0005
Year × population	1	6.84	3.10	0.1230
Number of pollen grains per tree (R^2 = 0.508)				
Year	1	120.42	56.22	0.0001
Population	1	29.26	22.48	0.0002
Year × population	1	7.120	3.92	0.1126
Number of pollen grains per microsporophyll (R^2 = 0.484)				
Year	1	52.12	28.12	0.2112
Population	1	14.10	4.40	0.0110
Year × population	1	4.94	1.32	0.2452

ering periods in 2000 and 2002. The data show that pollen frequencies near the source tree were highest in all directions. At a distance of 3 m from the source tree, the pollen frequencies were 95.0%, 96.5% and 93.3% towards uphill, downhill and horizontal dissections respectively. Pollen frequencies decreased sharply at the distance of 12 m from the source tree. At this distance the pollen frequencies remained half of the source frequency for uphill and horizontal directions. However, in the downhill direction, 52.0% frequency to the source frequency was observed at 24 m from it. Significant dispersion of pollen grains in different directions were: up to 48 m in the uphill direction, 192 m in the downhill direction and 96 m in the horizontal direction, and the mean pollen frequencies relative to the source frequencies at these distances were 14.2%, 3.8% and 7.2% respectively. In the uphill and horizontal directions pollen grains travelled only up to 96 and 192 m respectively, and the average pollen frequencies to the source frequency were 1.5% and 0.9% respectively (Figure 1). However, in the downhill direction pollen grains could migrate up to 768 m, but the pollen frequency relative to the source frequency at this distance was low, i.e. 0.2.%. The total pollen output per unit area (i.e. 1 cm^2) on a series of 10 pollen slides in the downhill direction was averaged at 1205 pollen grains. However, in the uphill and horizontal directions it was averaged at 927 and 1055 pollen grains on a series of seven and eight pollen slides respectively. Pollen dispersion distances are best expressed as the slope of the dispersion curve[17]. Therefore, the respective regression equations calculated for three different directions were: (i) $\log F = 2.3175^{-0.00307D}$ (uphill direction), (ii) $\log F = 2.3326^{-0.00400D}$ (horizontal direction) and (iii) $\log F = 2.3502^{-0.00382D}$ (downhill direction).

Phenological studies in natural stands at two different locations in 2000 and 2001 indicate that pollen-cone-bud initiation begins about mid-June, 14–16 weeks before pollen is shed; growth resumes about 6–8 weeks later, followed by 2–4 weeks of pollen development. Dehiscence begins about one week later at the higher altitudinal location (Ghimtoli) than at the lower altitudinal location (Teka). Pollen development and dehiscence are more temperature-dependent, and dehiscence is strongly influenced by relative humidity. Microsporangium dehiscence in *C. deodara* shows diurnal periodicity and is duly correlated with the diurnal alteration of temperature and humidity. High humidity and reduced temperature inhibit dehiscence during morning and evening hours of the day. When these two factors returned to more standard value at midday, maximum dehiscence was observed in both sites. It seems quite likely, therefore, that dehiscence is a desiccatory process, which confirms the findings of several workers[18–20]. Dehisced or split-open pollen sacs give us accurate estimates of the daily pollen release, which is a prerequisite for knowing the pollination system and breeding behaviour of any anemophilous species at a given place. In the management of seed orchards, knowledge of microsporangium dehiscence is essential to obtain temporal reproductive isolation, if needed, by inducing bloom delay in the desired trees.

Temporal and spatial variations for pollen production in *C. deodara* have been detected, which suggests that the climatic conditions play a significant role in this variation. It was also observed that flowering was totally absent in about 50% of trees at both sites in 2001, which suggests a two-year cyclic pollen yield in *C. deodara*. Different species had different intervals between bumper years of production[21], for example, a cycle of 5 years for *Quercus*, 2 years for *Pinus* and *Fagus*, and 3 years for *Fraxinus* and *Ulmus*. Pollination efficiency in anemophilous species largely depends on the concentration of airborne pollen. Low pollen production exhibits poor pollen dispersal, which ultimately affects ovule fertilization and seed production negatively. As in case of *Taxus canadensis*, Allison[22] reported that decreased pollen production in a natural population in one year, reflected reduced pollination success in that population. Therefore, it is most important to have an estimate of the total pollen production per plant, because the production of seeds often depends on production of pollen[23].

The level of pollen production per tree in *C. deodara* ranged between 1.9×10^9 and 21.6×10^9. However, in some other conifer species the figure reported was 20.9×10^9 to 32.3×10^9 for *Pinus pinaster*[13], $12.5–27.3 \times 10^{11}$ for *Pinus roxburghii*[24] and 1.31×10^9 to 1.74×10^{10} for Chinese fir[25].

Pollen trapping studies in *C. deodara* indicate that the concentration of pollen dispersed from isolated source tree drops off rapidly with distance, with highest densities within 50–100 m of the source, but small amounts travel up to several hundred metres away. The result suggests that most intermating within local populations occurs among neighbouring individuals. Nevertheless, when whole stands are considered as pollen source, the accumulated long-distance dispersal of small amounts of pollen from several trees can result in considerable pollen distributed over long distances[26,27]. Thus, the gene flow potential between nearby populations is high. The results of this study are well supported by those of other studies[8,28,29]. The quantity of pollen transported over long distances is small compared to the total pollen production by an individual. The frequency of airborne pollen declines rapidly as the distance from the source increases (Figure 1). This rapid decline is of great practical value in planning and managing a seed orchard. In *C. deodara*, a pollen frequency of 0.90% and 3.8%, relative to the source frequency was recorded in the horizontal and downhill directions respectively, at 192 m, which suggests that an isolation strip of 192 m may be considered minimal for managing a *C. deodara* seed orchard.

Topography seems to have an influence on the direction of pollen spread. Total pollen output in a series of different distances at different directions was minimum on the uphill sides and maximum on the downhill direction, which indicates the positive effect of gravity on the rate of fall of pollen grains. The presumptions of pollen dispersion patterns in wind-pollinated species are that most mating occurs among neighbouring plants, and does not take into account the influence of competing pollen sources[11]. If the amount of pollen produced by near neighbours is small, relative to more distant pollen sources, the advantage of proximity may be eliminated. Patterns of mating estimated from pollen dispersal alone also do not account for differential pollen fertility or floral phenology of potential male parents or their crossability with specific females[30].

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C. M. SHARMA[1]
V. P. KHANDURI[2,*]

[1]*Department of Forestry, HNB Garhwal University, Srinagar Garhwal 246 174, India*
[2]*Department of Forestry, Mizoram University, Aizawl 796 001, India*
**For correspondence.*
e-mail: khandurivp@yahoo.com