Pollen production, release and dispersion in Himalayan alder (*Alnus nepalensis* D. Don.): a major aeroallergens taxa

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Abstract

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Alnus nepalensis is a medium-sized, deciduous tree that occurs in the Indian sub-continent, South America, Hawaii, and China. It is a prolific pioneer species in freshly exposed soil in landslide areas of the western Himalayas and has the potential of fixing nitrogen. A study was conducted to assess the reproductive phenology, pollen production, pollen release, and pollen-mediated gene flow of *Alnus nepalensis* by considering a patch of trees as a pollen source in the temperate forest of Garhwal Himalaya to develop sustainable management strategies relating to the plantation geometry in seed orchards. Staminate flowers of *A. nepalensis* are composed of "cymules". The presence of bifid stigma and protandry condition were the unique features of the species. Flowering in the male phase was initiated in the last week of September and continued till November. Peak shedding of pollen generally proceeds peak receptivity by 1–2 weeks. The time between onset and peak flowering was 2 weeks 4 days and the total average duration of the flowering period was about 24.8 days. Temperature and relative humidity played a major role in pollen release and the maximum pollen release occurred at 29.2 °C at 13.00 hrs of the day. Pollen production per catkin varied significantly among trees. The average pollen grains per tree were 2.20×10^{10}. The pollen-ovule ratio suggests that the breeding system of *A. nepalensis* falls under the class xenogamy. Pollen mediated gene flow revealed that the significant pollen which can cause pollination of *A. nepalensis* can travel up to 40 m uphill and 80 m in downhill directions. Thus, an isolation strip of 80 m is sufficient to manage the seed orchard of *A. nepalensis* in the western Himalayan region.

Keywords

Introduction

Himalayan mountain ranges are diverse and globally important. It plays a significant role in contributing to the best ecosystem services (Sharma et al., 1992). Though these ranges are recognized among the most frangible mountain systems in the world and are now threatened due to continuous human intervention and climatic factors (Bargali, 2011). Recurring landslides during the rainy season have become a disastrous phenomenon in most of the hilly regions (Sharma et al., 2004). Frequent landslides and anthropogenic activities, i.e., deforestation, overgrazing, and forest fire (Skilodimou et al., 2018) lead to low concentration of nutrients like nitrogen bring changes in

the soil carbon structure (NEFF et al., 2005), increases concentration of noncombustible elements such as calcium, potassium, magnesium, and phosphorus (DIAGNEE et al., 2013; SHARMA et al., 1998) along with contaminating the soil with zinc and lead (MCGRATH and ZAHO, 2003). Thus, soil fertility is a major problem in hill forestry and agriculture systems. There's necessity of plantation of tree species particularly those that have the nitrogen-fixing ability, which is important for improving soil fertility.

Alnus nepalensis D. Don is considered to be the best for bio amelioration of the soils, because of the beneficial characteristics such as; the tree cover having more root-shoot biomass and leaf litter fall, extensive root system which increases soil organic carbon, better aggregation, improved water transmissivity, and infiltration, in turn, reduce soil erosion (SAHA et al., 2007). Due to the symbiotic relationship with nitrogen-fixing bacteria, i.e., Frankia, actinorhizal plants (SHARMA and AMBASHT, 1984; LUNA, 2005) forming 80 nodules per plant (POKHRIYAL et al., 1993) perform more efficiently in degraded land. A. nepalensis is a prolific pioneer species in freshly exposed soils in landslide-affected areas, and rocky and degraded slopes (SHARMA et al., 1998). A. nepalensis is a fast-growing tree (BARAKOTI, 2006) and has the potential to improve Zn, Pb, and Cd-contaminated soils showing the species' potential to reclaim polluted soil through the phenomena of phytoextraction of Zn, Pb, and phytostabilization of Cd (JING et al., 2014; RATHORE et al., 2010). Apart from these benefits, information about flowering phenology, pollen production, dispersal, and pollen gene flow of A. nepalensis is not available so far except for general taxonomic information (TROUP, 1921; ABBE, 1935; SCHOPMEYER, 1974; SHARMA et al., 1998; RATHORE et al., 2010; MUNRO, 2014). Alnus pollen is the major aeroallergen of the Northern Hemisphere (GHASEMIFARD et al., 2020).

The flowering time (when to flower) of each species is genetically fixed, which shows high variability with environmental factors, i.e., precipitation and temperature (AMASINO and MICHAELS, 2010, ROJO et al., 2021). Pollen production is the key attribute of reproductive success and fitness of natural populations (ALLISON, 1990; GÓMEZ-CASERO et al., 2004; LADEAU CLARK, 2006; KHANDURI et al., 2019a). The knowledge of pollen production per individual tree along with flowering phenology would be useful for estimating the pollen season to aerobiologists and tree breeders for making crosses for enhancement of wood quality through provenance testing and mating design, foresters, silviculturists and ecologists for natural selection and progression of plant life history (KHANDURI, 2014; KHANDURI, 2023).

Gene flow studies in plant species emphasized the role of pollen movement and the mode of pollination. The degree of the mode of pollination depends on the spatial distance between the individuals of species (BOHN et al., 2016). Gene flow is a key factor in the evolution of species, influencing effective population size, hybridization, and local adaptations (GERBER et al., 2014). Gene flow is important in shaping the genetic structure of the population and is a key factor for determining the composition of the ecosystem (KHANDURI and SUKUMARAN, 2019). Spatial restriction of gene flow can lead to non-random mating and the subdivision of a population into genetic neighborhoods (SCHAAL, 1980). Numerous studies on gene flow in plants have shown that pollen flow is the major contributor to the gene flow (ELLSTRAND et al., 1989; ELLSTRAND, 1992;

KAUFMAN et al., 1998; SHARMA and KHANDURI, 2007; BOHN et al., 2016; KHANDURI and SUKUMARAN, 2019; ZHANG et al., 2019). For wind-pollinated trees, pollen-mediated gene flow can be considered the most important mechanism to maintain genetic connectivity over long distances (BEGHE et al., 2017). Wind-borne pollen grains of widely distributed tree species have the potential to travel dozens or hundreds of kilometers (BOGAWSKI et al., 2019; PICORNELL et al., 2020).

Genetic differences between individuals are crucial for the adaption and evolution of a population as new gene combination is generated by the recombination process, mating between unrelated individuals increases genetic variability (BONA et al., 2019). Any differences between populations that cannot be explained by locally selected adaption must have evolved by genetic drift (ELLSTRAND, 2003). Gene flow within and between plant populations has been of continuous interest to plant breeders and seed producers for many decades. Economic consideration has stimulated studies of gene flow as a function of distance, breeding system, pollinating agent, and planting design in numerous domestic plants (LEVIN and KERSTER, 1974). Long-distance dispersal events are very important in colonization and in breaking down population isolation (GILLESPLE, 2012). The Alnus species are generally planted throughout the Himalayan zone. It is important to properly assess how the microclimate of an area affects its phenology and pollen distribution. Keeping in view the above facts and problems, this study aims to develop strategies relating to plantation geometry through the assessment of pollen production, pollen-mediated gene flow, and pollen dispersion by considering a patch of trees as a pollen source for sustainable management of seed orchards in Garhwal Himalaya. The objectives of the study were: (i) To assess the phenology, extension of the catkins, anther dehiscence, and pollen dispersion in the air concerning time and temperature of the day, (ii) Estimation of variation in pollen output per anther, catkin and per tree, and (iii) Pollen mediated gene flow by considering a patch of trees as a pollen source.

Materials and methods

Study area

The study was carried out on the premises of the College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand on the way to Dandachali Road from August 2019 to December 2019. The study site lies between 30°18"N and 78°24"E with an altitude of 1,600–2,192 m above sea level (asl). The soil of the study area is silty clay loam, brownish-black in colour, coming under the series of orthents (Entisol). The pH of soil ranges between 5.3–5.6. (KHANDURI et al., 2019b). The important tree species of the area is mainly consisted of *Pinus roxburghii, Pinus wallichiana, Cedrus deodara, Quercus leucotrichophora, Lyonia ovalifolia, Myrica esculenta, Prunus cerasoides, Pyrus pashia, Rhododendron arboreum*, etc. The study area is in the mid-hills of the Himalayas and typically experiences a moist temperate climate with chilled winters. According to the Koeppen's classification, the Uttarakhand's mountainous terrain is categorised under the climate type "Et" where the average temperature ranges from 0–10 °C and rainfall

Table 1. Monthly meteorological data during 2019

Month	Tmax (°C)	Tmin (°C)	Rainfall (mm)	RH1 (morning)	RH2 (afternoon)	Wind speed (kmph)	Sunshine (hours)	Evaporation (mm)
Jan	10.5	0.1	98.6	79	63	4.6	5.1	1.0
Feb	11.3	1.5	136.8	84	70	4.9	4.5	0.9
Mar	15.9	4.4	40.9	73	52	4.8	7.2	2.0
Apr	22.4	10.0	22.5	66	50	4.1	8.7	3.5
May	26.0	12.1	21.3	53	35	4.3	9.3	4.4
Jun	27.7	14.3	39.5	64	50	3.9	9.0	4.6
Jul	23.4	15.3	175.6	93	82	2.0	4.2	1.9
Aug	23.4	15.4	172.2	95	81	2.6	4.2	1.8
Sep	22.6	14.1	139.1	95	79	3.8	5.1	1.7
Oct	20.0	8.8	27.9	85	68	2.8	7.1	1.5
Nov	17.5	6.1	27.5	77	63	3.9	6.1	1.4
Dec	12.8	0.6	61.1	78	62	4.1	6.2	0.8

RH, relative humidity (%).

fluctuates yearly. The average rainfall received during the study period (year 2019) was 85.56 mm experiencing the highest rainfall in August, i.e., 172.2 mm, and the lowest in November, i.e., 27.5 mm. The recorded mean monthly maximum (19.26 °C) and minimum temperature (9.0 °C) along with relative humidity (RH), wind speed, sunshine, and evaporation for the year of the study, i.e., 2019 are presented in Table I. Snowfall occurs in the months between mid-December and mid-January.

Study species

The trees studied in the present investigation were wild individuals that were present in the natural population. *A. nepalensis*, belonging to the family Betulaceae, also known as the "Birch family", is a native of the Himalayas, commonly known as Himalayan Alder, Nepalese alder (SHARMA et al., 1998), Indian alder in English, Utis in Hindi (TROUP, 1921; LUNA, 2005). It occurs in the hills of the Himalayas around 2,000 m amsl (TROUP, 1921; LUNA, 2005). About 35 species of *Alnus* are reported (SHARMA et al., 1998) which are an important group of trees not only in the Indian sub-continent but also in South America, Hawaii, and China. *A. nepalensis* prefers moist climates with a mean annual temperature of 13–26 °C (CHATURVEDIET al., 2018), a shady ravine near water, though it can withstand imperfectly drained areas and flooding but cannot withstand water logging. It shows the best performance on well-drained deep loamy alluvial soils but can grow on a wide range of soils from gravel, sand, and clay (TROUP, 1921; LUNA, 2005; RATHORE et al., 2009). It is a wind throw species when grown alone, a good coppice, and has the ability to compete with the weeds (LUNA, 2005; JING, 2014). It is also used as a commercial timber production due to moderately soft wood and charcoal feedstock. Moreover, it is also a chief associate in various commercial timber plantations, where it helps to raise the nitrogen levels of fields (LUNA, 2005). *Alnus nepalensis* has the potential of yielding a red dye and tannin from its bark (GAUR, 2008). It is a pioneer species in the landslide areas of the Himalayan region (CHAMPION and SETH, 1968) and regulates many ecological services such as soil development, regulation of nutrient and water cycling, and forest structure development.

The most prominent feature of the flower of *A. nepalensis* is the presence of compact aggregate clusters of small yellow flowers or staminate clusters. Catkins hanging in the cluster at the end of the twigs, grouped in a terminal panicle separate or on the same twigs, which flowers from September to November, pollinated through the wind (anemophilous pollination) (SCHOPMEYER, 1974). Female catkin or cone appearance strobili grouped in a short axillary raceme of 3–8 catkins, rarely solitary occurring on the branching side of the twigs (TROUP, 1921; SHARMA et al., 1998; RATHORE et al., 2010). The staminate flower is unisexual, with compound spikes, and the partial inflorescence is reported as "dichasium" or a "cymule" (three-flowered) with a sessile flower. The pistillate inflorescence is composed of the large number of cymules arranged helically on the primary ament axis (ABBE, 1935) in each pistillate cyme, there are primary, secondary, and tertiary bracts forming a "bract complex" (ABBE, 1974). Fruits which resemble the cone of the pine family are dark brown upright on short stalks, elliptical, composed of many hardwood scales (RATHORE et al., 2010), and small winged fruits or nuts (GRIMM and RENNER, 2013). The empty catkins remain on the tree for a long time which is the most distinguishable feature of the alders to the birches (TROUP, 1921). Seeds are light brown, circular, and flat with two board membranous wings nutlets or short-winged called samara (MUNRO, 2014).

Morphometric observations

Trees containing clusters of male catkins and female cones of *A. nepalensis* in a natural population were chosen randomly prior to bud initiation. Observations were recorded weekly from August 2019 to November 2019 on clusters of male catkins and female cones. The different stages of development were recorded with the help of a hand lens.

The morphological parameters such as type of inflorescence, and the initial and final length of the male catkins and female cones were taken with the help of a scale, and the date of elongation of male catkins and the date of expansion for female cone buds were recorded. The shape of the male catkins and female cones and the colour changes on the male catkin and female cone were closely observed daily with the help of a hand lens to check the stigma development and recorded its time duration for anthesis. The number of cymules per catkin for males and scales per ovule for females was counted for 100 randomly selected male catkins and 100 female cones. The number

of catkins per cluster and number of cones per cluster were counted for 20 clusters, diameter of female cones was taken every two weeks with the help of a Vernier caliper. To elucidate the anther structure and morphological changes of the stigma, more catkins and cone clusters were collected within the weeks and observed under a stereoscope with the help of a 20× lens. The flowering phenological data were observed in terms of; i) duration of the first and last flowering, ii) peak flowering time, and synchrony (flowering overlap among individuals).

Pollen release in relation to diurnal temperature

A passive method of aerobiological monitoring was used to study the pollen release in relation to diurnal temperature. At the time of peak blooming, microscopic slides coated with petroleum jelly (Vaseline) were kept to track the pollen release. The microscopic slides acted as pollen traps for every two hours intervals (i.e. 7.00, 9.00, 11.00, 13.00, 15.00, and 17.00 h) and the temperature was recorded in each interval with the help of a digital thermometer. Three microscopic slides (T1, T2, T3) in each interval were mounted vertically on the wooden sticks or either placed perpendicular to the prevailing wind direction. It was ensured that the slides were kept at the height corresponding to the height of the catkins and also 1.5 m height above the ground. Slides were replaced at every two hours intervals and the number of pollen grains per slide was counted in a 1 cm^2 area under a binocular microscope therefore, the unit used to determine the pollen release was total pollen cm^{-2}. Pearson correlation was calculated between temperature and the number of pollen released at different times.

Pollen production

Five trees were selected for the estimation of pollen production. At first, the number of primary branches was counted, and then secondary branches with male catkins and female cones were counted in each tree. Further, a random sample of 20 mature male catkins was collected from each tree (i.e., $20 \times 5 = 100$) and the number of cymules was counted manually. Similarly, anthers per cymule were counted manually. The undehisced anthers were viewed under a stereo microscope and obtained from the cymules (anthers were removed with the help of needles) and counted under the binocular microscope. Due to the small size and large number of pollen grains, the anther was placed at the center of the slide, a small drop of water was added and the anther was squeezed and crushed with the help of needles and forceps to release all grains on a microscopic slide, the debris of anther on microscopic slides were removed gently. The pollen grains were counted using a binocular microscope. The number of pollen grains was counted for the top, middle, and bottom microscopic views (1st, 2nd, 3rd) by moving the slide from one end to the other and then adding all the microscopic views. The method used for pollen productivity analysis was modified after Tormo-Molina et al. (1996) and Khanduri et al (2019a). To estimate the total production of pollen grains per tree, the following equation was used: Pollen production per tree = number of anthers per tree × average number of pollen grains per anther; Number of anthers per tree was calculated as number of primary branches with catkin clusters × average number of secondary branches per primary branch × average number of catkin clusters per secondary branch × average number of catkins per catkin cluster × average number of cymules per catkin × average number of anthers per cymule. Pearson correlation between the number of catkins per tree, number of cymules per tree, number of anthers per tree, and total ovules per tree with tree height and diameter was established. ANOVA with two factors was performed by using JMP statistical software pro-14.

Total ovule production and pollen ovule ratio

Five trees that were selected for the estimation of pollen grains were also selected for the estimation of ovule production. The tree selected for the study was phenotypically superior and the distance between the trees was 300 m. The average height of the tree was 11.9 ± 1.32 m and the diameter was 71.4 ± 15.07 cm. These trees were growing in the eastern aspect in sloppy land and the soil of the area was acidic. At first, the numbers of primary branches were counted and then secondary branches with female cones were counted in each tree. Further, a random sample of 20 mature cones was collected from each tree (i.e., $20 \times 5 = 100$). The number of ovules were determined by counting them on scales for twenty cones on each tree (i.e., $20 \times 5 = 100$). Total ovules per tree were obtained as; number of primary branches × average number of secondary branches per primary branch × average number of cones on a secondary branch × average number of scales per cone × average number of ovules per cone. Pearson correlation was established between the number of cones per tree, the number of scales per tree, and the total ovule per tree with tree height and diameter. The pollen-ovule ratio was determined by dividing the estimate of the number of pollen grains per flower (catkin) by the number of ovules per flower (cone) as per the method of Cruden (1977). The ratio was derived from the mean pollen quantity with the mean ovule's quantity.

Pollen mediated gene flow

Pollen mediated gene flow was recorded in two directions from the source trees (uphill direction and downhill direction. 1) Five isolated trees were selected as pollen sources from which pollen-mediated gene flow was carried out in an uphill direction up to 80 m and downhill direction up to 10 m because of the unfavorable geographical conditions. 2) The same experiment was conducted up to 80 m towards the downhill direction considering thirteen groups of trees as pollen sources (focal trees) located on an isolated patch. Measurement of height was taken with the help of Ravi altimeter and diameter of breast height was measured with the help of tree caliper of focal trees. For both directions, the pollen source trees were in one patch and isolated from other possible pollen contamination from foreign pollen and there were no other flowering trees from other pollen sources. Petroleum jelly-coated microscopic slides were used as pollen traps. The slides were mounted vertically on wooden rods and placed perpendicular to the direction of the prevailing wind around the source tree at geometrically increasing intervals (i.e., 0 m, 5 m, 10 m, 20 m, 40 m, and 80 m) as per the method used by Sharma and Khanduri (2007) and Adams-Groom et al. (2017). Three pollen slides (T1, T2, T3) were placed between 9 am and were collected after 24 hr during the peak flowering period. The pollen

count was made directly from the exposed glass slides (area 1.0 cm^2) under the binocular microscope. The effect of distance and direction on pollen dispersal was tested with regression analysis. Pollen dispersal was modeled as a function of different directions, and the distance from the source tree using regression analysis with pollen grains per slide was taken as the dependent variable, with distance from the source tree as the independent variable.

Results

Floral morphology structure and development

The advent of the bud bursting of male catkin occurred in the first week of August. At the initial stage, the male catkin was tender, short, and erect with an orange-like tinge at the top which disappeared with the increase in length. At the initial stage, the catkins were called spikes. At about 0.5 cm in length, the male catkins developed a pink feather-like structure giving the whole catkin a dusting brush-like look, and an average of 15.8 catkins per cluster produced which eventually turned yellow in colour. The catkins were erect and highly conspicuous and spiked until the third week of September, drooping of the spike was seen in the last week of September with cymules becoming clear and distinct and started losing white powdery and silvery colour and an average number of cymules per catkin was 113.92. Before anthesis, the male catkins partially segregate and the anthers turned red in colour. The cymules start unfolding and arranging themselves in a thread-like structure and turned yellow colour and bursting occurred. Thus, the final anther dehiscence occurred. The length of the catkin before elongation was 3.98 ± 0.17 cm, while after complete elongation of catkins, the length was 10.02 ± 3.02 cm.

During the study period, it was observed that the female cones were erect, spherical, and highly compact and occurred in the raceme pattern with whitish stigma and scales curved at the top forming a condensed spherical cone or strobili. The female cones were dark green in colour, comprising scales. A single scale bearing two ovules, which became more spherical during the growing period and the final diameter was 2.13 ± 0.12 cm and length was found to be 1.83 ± 0.35 cm. The cones varied in shape, size, and thickness and an average of 5–6 cones were found in a cluster. The change in colour of the female cone was seen 1–2 weeks after the blooming of the male part (i.e., catkins). However, synchrony in pollen shed and female receptivity was also observed. Thus, the average date of the first flowering was observed in the first week of October. The purplish colour was seen in the tip of the scales which indicates the extension and maturity of the bifid stigma, opening of the cones, ripening of the cones, and stigma receptivity of the female part. The change in colour of the stigma was also seen from white to red and the declined receptivity was reflected through the stigma forming an arc in the scales. In the first week of November, the cones changed into a hard woody structure thus giving the advent of the seed set. *Alnus* fruits were small, smooth surfaced, light, dry, indehiscent, and laterally winged the summit of the fruit is crowned by two persistent styles and is termed samara, the dispersal of the seed occurred through the wind. The empty cone remains attached to the tree even in the second year also.

Flowering phenology

During the study period, it was observed that flowering in the male phase started in the last week of September and continued till November. A synchronisation in flowering was seen within and among the individual trees. The time between onset and peak flowering was 2 weeks 4 days and the total average duration of the flowering period was 24.8 days (Table 2) representing a synchronisation pattern of flowering among individual trees. Protoandry was the unique feature of the species. The opening of the female cones occurred 1–2 weeks late for the male flowering. It was also observed that female cone opening occurred after the peak flowering.

Table 2. Variation in the phenophases of male catkins of *Alnus nepalensis*

Observed variable	Average date
First flowering	5 October
Peak flowering	20 October
Last flowering	29 October
Flowering duration	24 August

Pollen release in relation to diurnal temperature

No pollen release occurred early in the morning at 7.00 hrs with a temperature of 17.5 °C. The proportions of pollen release increased with the increasing temperature and peaked at around 13.00 hrs (66.33 ± 5.51) at a temperature of 29.2 °C, followed by 15.00 hrs and 11.00 hrs with the temperature of 22.1 and 24.1, respectively (Fig. 1). The second highest value of pollen release with temperature 22.1 is due to the lower relative humidity in the afternoon (Table 1). Thus, reflecting that a suitable level of temperature and humidity play an important role in the subsequent release of pollen grains. A strong positive correlation of 0.8976 was also observed with the temperature of the day. This gives important information about how abiotic factors and microclimatic conditions are directly proportional to pollen release.
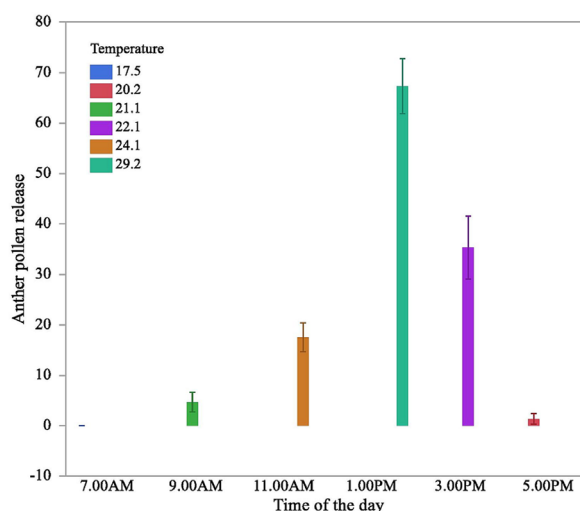

Fig. 1. Pollen release in *Alnus nepalensis* in relation to time and temperature of the day (each error bar is constructed using ± Standard error).

Table 3. Variation in production of male and female gamete per tree

Serial number	Observed variables	Average production ± SD
1.	Tree height (m)	11.9 ± 1.32
2.	Tree diameter (cm)	71.4 ± 15.07
3.	Number of Primary Branches	13 ± 2.82
4.	Number of secondary branches per Primary Branch	6.87 ± 0.40
5.	Number of catkin clusters per secondary branch	4.73 ± 0.30
6.	Number of catkins per catkin cluster	15.8 ± 1.75
7.	Number of cymules per catkin	113.92 ± 6.39
8.	Number of anthers per cymule	6 ± 0.0
9.	Number of anthers per tree	$4.63 \times 10^6 \pm 1.13 \times 10^6$
10.	Number of pollen grains per anther	4,774.13 ± 143.28
11.	Pollen grains per catkin	$3.27 \times 10^6 \pm 0.22 \times 10^6$
12.	Total pollen grains per tree	$3.14 \times 10^{10} \pm 0.60 \times 10^{10}$
13.	Number of cones (female) per secondary branch	3.07 ± 0.22
14.	Number of scales per cone	119.7 ± 5.87
15.	Number of ovules per cone	218.81 ± 9.53
16.	Total ovule per tree	75,824.78 ± 16,549.24
17.	Pollen ovule ratio	14,926.81 ± 894.55

Pollen production

In *A. nepalensis*, the number of anthers per tree, the number of pollen grains per anther, pollen grains per catkin, and the total pollen grains per tree varied from tree to tree. The average number of anthers per tree was observed as $4.63 \times 10^6 \pm 1.13 \times 10^6$ anthers. Total anthers per tree ranged between 1.11×10^6 and 8.08×10^6. Pollen grain per anther ranged between 4,430 and 5,146 with an average of 4,774 ± 143.28. Moreover, the average pollen grain per catkin was found between 2.52×10^6 and 3.84×10^6. The total pollen grain per tree was $3.14 \times 10^{10} \pm 0.60 \times 10^{10}$ (Table 3).

Ovule production and pollen ovule ratio

The average number of cones developed on secondary branches was 3.97 ± 0.50. The average number of cones produced per tree was 376.24 ± 137.51. The total ovule production per tree was estimated as 8,2324.40 ± 3,7030.30. Pollen grains per ovule were estimated as 14,913.46 ± 2,003.79 (Table 3). The difference in pollen production per anther and ovules per cone between trees differs significantly. Also, the pollen-ovule ratio among trees was significantly different. Based on pollen ovule ratio indices of CRUDEN (1977), *A. nepalensis* falls under xenogamy, i.e., out-breeding system.

Statistical results

The trend between anther dehiscence and diurnal temperature resulted from the best polynomial fit of degree 2 with the temperature having coefficient of determination of 83.43% (Fig. 2) and the equation appeared as:
Anther dehiscence = $-98.8137 + 5.1795518 *$ Temperature $+ 0.3008822 *$ (Temperature $- 22.3667)^2$.

The total production of catkins, cymules, anthers, pollen grains, cone scales, and ovules per tree are correlated as one is multiplied by the next to obtain the total production per tree. The height and diameter of the tree had a positive correlation with the number of catkins, cymules, anthers, pollens, cone scales, and ovules per tree due to
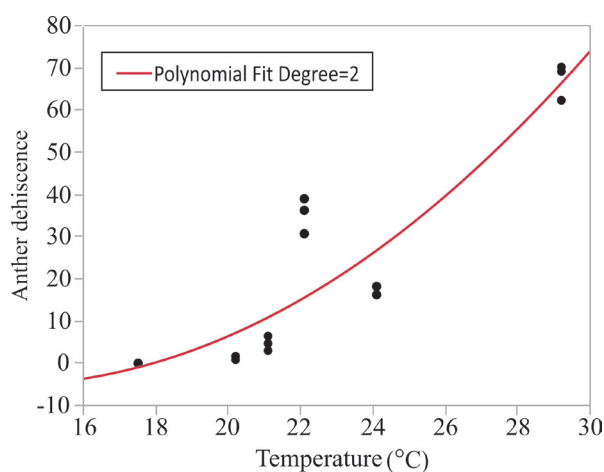

Fig. 2. Bivariate fit of anther dehiscence by temperature (°C).

comparatively moderate height which leads to higher production. It is a known fact that anemophilous plants are characterized by high pollen production. But the wind being the vector of pollination for these plants effects the pollen efficiency. The unspecific nature of wind sometimes compensates for the reduction of pollen efficiency. For the tree height, moderately positively correlated variables were apparent, such as the number of catkins per tree (r = 0.58), number of cymules (0.64), anthers (0.64), pollen grain per tree (r = 0.64), number of cones per tree (r = 0.63). On the other hand, a moderately positive correlation was observed for tree diameter with the number of catkins, cymules, anthers, the number of pollen grains (r = 0.44, 0.50, 0.50, 0.52, respectively).

Relation between tree height and diameter for parameters, such as cone per tree, scales per tree, and ovule production per tree were moderately correlated, i.e., cone per tree (r = 0.63), number of scales per tree (r = 0.61). The total ovules per tree reflected a weak positive correlation (r = 0.21) with tree height. However, a moderate correlation was observed for diameter with scales, and ovules per tree (0.42 and 0.44, respectively).

The results of the analysis of variance indicate that

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there is significant (p < 0.01) variation among individuals for pollen production per tree. However, variation among the pollen production per branch was found to be non-significant.

Pollen mediated gene flow

Pollen dispersal at two different directions was not uniform and varied with different distances from source trees. Results show that the frequency of pollen flow was increased with increasing distance up to 10 m from source trees in uphill and downhill directions. Maximum average pollen grains were found at a 10 m distance from source trees, i.e., 44.00 ± 6.00 per cm^2 in the uphill direction and 139 ± 17.2 per cm^2 for the downhill side. Pollen grains can travel up to 80 m distance from source trees towards downward directions, (1.3 ± 0.6 pollen grains per slide). However, in uphill directions, it can travel up to 40 m only (Fig. 3).
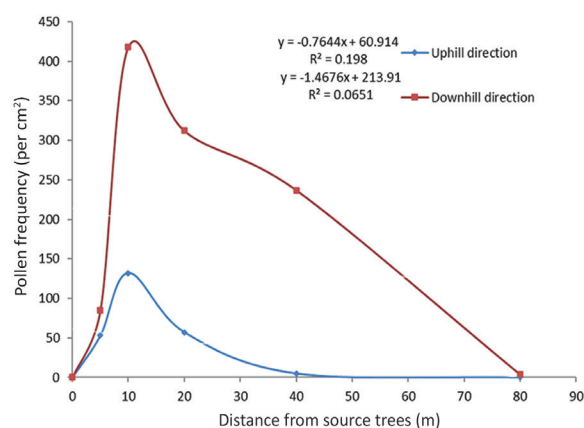

Fig. 3. Pollen migration in *Alnus nepalensis* towards uphill and downhill directions from source trees.

Discussion

Flowering phenology

Accurate phenology assessment allows us to better understand population dynamics and picking of provenance that is ideally suited to new climatic conditions (Cleland et al., 2007). The flowering phenology of *A. nepalensis*, according to Luna (2005) is from September to October. In *Alnus glutinosa* male and female catkins develop in the autumn of the preceding year and appear in the subsequent spring (Houston et al., 2016). While *Alnus incana* produced flowers earlier than leaves opening in late February to May (Houston et al., 2016). In comparison, the current study found that the male phase of flowering began in September and lasted until November. The male flower cycle started in August when bud bursting happened 20 days after the initiation and buds remained erect for 40 days and drooping of catkin started after 11 days. Similarly, cone initiation started in August and enlargement took 30 days. The average duration to complete the reproductive cycle was 2.5 months. Peak shedding of pollen precedes peak receptivity but synchrony in receptivity and pollen shedding is also seen during the study period. Similar results have also been reported by Brown (1995) in *Alnus ru-*

bra. Moreover, the present study reveals that *A. nepalensis* flowered for approximately 3 weeks in the study area which is well supported by the other study in *Alnus taxa* (Dabrowska et al., 2012). According to Fashel and El-Kassaby (1987), the prolonged pollination period increases the chance of selfing. Nicholls and Dorken (2012) pointed out that in monoecious species geitonogamous selfing occurs. However, synchronous and asynchronous flowering has an impact on reproductive success which determines the level of outcrossing and selfing. Asynchronization in flowering was seen within and among individuals. Asynchronization has been shown to harm on random mating and minimizing selfing (El-Kassaby, 1987). The unique feature studied about *A. nepalensis* was the protandrous condition. The opening of the female cones occurred 1–2 weeks after male flowering. Protandry (pollen grains dispersal happens before the pistil becomes receptive) is also reported in *Alnus firma* and *Alnus sieboldiana*, avoiding self-pollination (Sogo and Tobe, 2005).

Pollen release in relation to diurnal temperature

The broad reflection of the aerodynamics requirement that leads to efficient pollen liberation from anthers and capture by stigma has been discovered in studies on structural and functional aspects of flowers of wind-pollinated plants (Shivanna and Tandon, 2014). It was clear from the observations that the pollen release depends on climatic factors, i.e. temperature and humidity. The optimum temperature required for *A. nepalensis* to release pollen was 30 °C. Pollen release is mainly controlled by the temperature in most anemophilous trees (Pacini and Hesse, 2004). The present study reflected a strong positive correlation of 0.8976 between air temperature and pollen release. Similar observations were also observed by several researchers (Jato et al., 2002; Dabrowska-Zapa et al., 2018; Ziska et al., 2019). Interestingly, pollen from *Alnus* trees is one of several sources of the annual atmospheric aerosol (Alvarez-López et al., 2020) and identified the *Alnus* as one of the most important allergic taxa, observing an increase in the incidence of pollen allergy diseases over the last half-century, which is the key biological contamination episode caused by the aeroallergens of the major allergenic tree species in environments. *Alnus* pollen has been reported as one of the major causes of pollinosis in Central and Northern Europe (D'Amato et al., 2007). Nevertheless, Smith et al. (2014) and Rojo et al. (2020) reported that the *Alnus* pollen is not a major allergenic pollen in the atmosphere of the Northern Hemisphere, at least in Europe. Pollen grain occurrence in the atmosphere is closely related to meteorological influences and the average daily temperature is considered a significant factor for pollen grain occurrence in the atmosphere (Bartková-Ščevková, 2003).

Pollen production

Anemophilous trees produce a large amount of pollen to ensure successful pollination in the long-range distance and the amount of total pollen production per tree ensures successful seed production which is important for the reproduction and development of plant community (Fageri et al., 1989; Alison, 1990). The total pollen production is influenced by various factors and the initial study on pollen production in anemophilous trees was done by Pohl

(1937) and gave the value of 2.52×10^6 to 3.84×10^6 pollen grains per catkin for *Alnus incana*. Further, ERDTMAN (1945), reported per catkin value (41×103) for *Quercus sessilifora*. MOLLINA et al. (1996) reported 340×10^3 to $1,100 \times 10^3$ per catkin for *Quercus rotundifoila*. In the present study, the total pollen grains per tree were $3.14 \times 10^{10} \pm 0.60 \times 10^{10}$. An analysis of pollen production in six oak species by FERNÁNDEZ-GONZÁLEZ et al. (2020), discovered that the average pollen amount per anther ranged from 3,400 pollen grains in *Q. robur* to 6,282 pollen grains in *Q. ilex*. The number of pollen grains produced per catkin varied between 355,237 and 716,385 for *Q. suber* and *Q. rubra*, respectively. Finally, pollen grains per tree varied between 436 billion and 1.6 billion for *Q. robur* and *Q. ilex*, respectively. GÓMEZ-CASERO et al. (2010) studied pollen biology in four Mediterranean *Quercus* species for two years. They discovered that the production of pollen grains per tree in the years 2000 and 2001 for *Q. suber* was $24,046 \times 10^6$ and $55,045 \times 10^6$, *Q. ilex* ssp. *ballota* $11,919 \times 10^6$ and $24,500 \times 10^6$, and *Q. faginea* $16,715 \times 10^6$ and $22,731 \times 10^6$ pollen grains per tree, respectively. In other cross-pollinated species, such as *Acer negundo* produces 17,374–96,520 pollen grains per anther and per tree, it ranged between 15.2 to 250.1×10^9 while, *Populus* produces 564.5 to 712.4×10^6 pollen grains per tree (MOLINA et al., 1996).

The height and diameter of a tree possess a moderate correlation with the number of catkins per tree, the number of cymules, anthers, and pollen grain per tree, and the number of cones per tree indicates that with the increasing height and diameter of the tree, number of catkins, cymules, anthers, and pollen grains per tree increased. On the contrary, the height and diameter of *Pinus roxurghii* have a negative significant correlation with pollen grains per tree (KHANDURI and SHARMA, 2002a), and the height of the tree in *Populus* and *Acer negundo* have a negative significant correlation with pollen grains per tree (MOLINA et al., 1996). The non-significant effect of tree sizes on male cone production and the significant effect on female cone production was reported in *Cedrus deodara* (KHANDURI et al., 2021). In a detailed study of six oaks species, negative correlations were found between pollen grains per anther, the number of anthers per flower, and the number of flowers per catkin. A positive correlation was recorded between pollen grains per anther and pollen grains per catkin. The number of anthers formed per flower was positively correlated to the number of catkins per tree and pollen development per tree. Pollen grains per catkin were also positively correlated to the number of flowers per catkin (FERNÁNDEZ-GONZÁLEZ, et al., 2020).

The amount of ovule production by an individual tree in a population varies from tree to tree. The total ovule number is an important factor affecting the seed set (STRELIN and AIZEN, 2018). In the present study, the relation between the total ovules per tree with tree height reflected a weak positive correlation while a moderate correlation was observed with diameter. Pollen-to-ovule (P:O) ratios have been commonly measured by plant population biologists and have often been related to the mating system, despite ongoing controversies concerning their indicative values. Within phylogenetic lineages, the ratio of pollen grains to ovules per flower may indicate the mating mechanism (CRUDEN, 1977; MICHALSKI and DURKA, 2009). As per the pollen ovule ratio, *A. nepalensis* was an obligate out-crosser. The *in vitro* pollen germination tests are generally used to evaluate the percentage of pollen that germinates, but they can also be used to measure pollen vigour by monitoring the rate of germination over time or the length of pollen tubes (SULUSOGOLU and CAVSOLGLU, 2014). HAGMAN (1969, 1970) conducted self-pollination and cross-pollination experiments with *A. incana*, *A. glutinosa*, and *A. incana × glutinosa* and assessed the pollen-tube development and seed fertility. Selfing of these species pointed towards good germination of pollens, with a few exceptions where pollen-tube growth retarded in style. Although, a considerable difference between various years was also observed. The pollen tube development was found intermediate between selfing and intra-specific crosses after interspecific pollination. These observations recorded by Hagman revealed the gametophytic type of self-incompatibility mechanism in *Alnus*. In this study, the acetocarmine staining method (ALEXANDER, 1969) was used, and it successfully stained all pollen. When pollen viability was tested using Trypan blue in *Betula pendula*, a similar result was obtained (DAFNI and FIRMAGE, 2000). It was also found that the pollen viability of *A. nepalensis* depends on genotype, pollen collection time and medium component, site conditions, etc.

Pollen mediated gene flow

The results for pollen-mediated gene flow for *A. nepalensis* indicated that the magnitude of pollen dispersed from the source tree in both directions, i.e., the uphill and downhill sides was not uniform. Notably, pollen from trees on the uphill side capturing was isolated, while on the downhill side, a patch of 13 trees was considered. Pollen flow frequency increased with the distance up to 10 m, then gradually decreased for both directions. However, the maximum pollen travel distance for isolated trees was 40 m, and for trees in patches was 80 m. Similar results were found for Douglas fir where the pollen dispersal range in pair trees was more than that of a single tree source with a maximum pollen count at a 15 m distance (SILEN, 1962). This result proposed that four main factors influence pollen dispersal distance: gravity, wind speed, direction, and species distribution pattern (DI-GIOVANNI and KEVAN, 1991), which is consistent with our findings. Similar results on pollen dispersal have also been reported in several studies (WRIGHT, 1952; BRAMLETT, 1981; SHARMA and KHANDURI, 2007; KHANDURI and SUKUMARAN, 2019). Based on the pollen trap technique, it was observed that the density of pollen decreases rapidly with the distance from male trees. Similar pollen dispersal frequency was also observed for some anemophilous trees at various distances from the source trees e.g., *Myrica esculenta* 110 pollen grains per slide at 64 m from the source tree (KHANDURI and SUKUMARAN, 2019) and 3 pollen grains at 45 m, 8 pollen grains at 100 m, 2 pollen grains at 45 m, respectively for *Pseudotsuga taxifolia, Populus deltoides, Fraxinus Americana*, respectively (WRIGHT, 1952). Similarly, the frequency of pollen grains relative to the source frequency for *Cedrus deodara* at 190 m was 2.5–5% (KHANDURI and SHARMA, 2002b), and in *Pinus roxburghii*, 2.16% at 640 m (KHANDURI, 2019). In *Malus sylvestris*, the average (50 percent) pollen flow distance was approximately 23 m, with a maximum distance of 60 m (LARSEN and KJÆR, 2008). In the present study, the effect of direction was also observed

for pollen dispersal. Total pollen count at different distances and directions was minimum for uphill and maximum for the downhill side. It indicated a free flow of pollen on the downhill side. Pollen dispersion studies are important in the conservation management of forest tree species because they promote good natural selection efficiency by allowing the population to remove undesirable pollen from the outside, which would be useful in the establishment of seed orchards (Larsen and Kjær, 2008). Thus, an isolation distance of 80 m may be considered best for the management of the seed orchard of *Alnus nepalenisis* in the western Himalayan region.

Conclusions

Reproductive phenological study of several temperate pioneer species is still lacking. *Alnus nepalenisis* being a good pioneer species with an ability of symbiotic relationship with nitrogen-fixing actinomycetes of the genus *Frankia* is considered a suitable species to improve degraded and landslide-prone land. Thus, this tree species is a potential soil improver and also adds considerable quantities of nutrients through the litter. The value of *Alnus nepalensis* has increased substantially in recent years and interest in the management of the species has increased accordingly, which in turn has led to an increased need for detailed information on the reproductive biology of the species for future silvicultural and management practices. The results obtained in the present study related to the timing of anther dehiscence and female receptivity may be used for making successful crosses to improve the quality of wood by selecting superior genotypes through screening of several populations so that a good seed orchard can be established for getting quality seeds for plantations of landslides prone areas in western Himalaya. The present study reflected a positive correlation of 0.8976 between air temperature and pollen release. Further, the information gathered on flowering phenology, pollen release, and pollen production under the present investigation could be used for the prediction of the level of pollen allergy during a particular period of the year. The pollen dispersion distances have immense practical value for detecting the isolation distances to establish the seed orchard. In our study, the maximum pollen travel distance for isolated trees was 40 m, and for trees in patches was 80 m suggesting that the isolation distance of 80 m may be considered suitable for the management of *Alnus nepalensis* seed orchards (a distance used for preventing seed orchards from undesired foreign pollen).

Author's contributions

AB and VPK conceived the study; AB set up the experiment, conducted fieldwork, and drafted the initial manuscript; VPK supervised and revised the drafts. BS set up the experiment, data analysis, MKR data analysis, statistical analysis, KSK and DR data analysis, and input in the initial draft. All authors read and approved the manuscript.

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