ing its formation could spell the success of a two-phase culture for optimum growth and maximum production in *C. fusiformis* mycelium and the economic extraction of the clavine alkaloid.

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Ecology of microsporangium dehiscence and pollen flow in Himalayan long-needle pine (*Pinus roxburghii* Sargent)

V. P. Khanduri and C. M. Sharma

Department of Forestry, HNB Garhwal University, Post Box 76, Srinagar Garhwal 246 174, India

Effect of temperature and relative humidity on microsporangium dehiscence and receptivity of megasporophylls in *Pinus roxburghii* were analysed. Ovulate strobili remained receptive up to five days with optimum receptivity between 1200 and 1600 h.

POLLINATION is an effective process of transfer by pollen vectors from the male to the female flower/organ, dependent primarily on air temperature and humidity. Wind is an important pollen vector and efficiency of wind pollination is generally believed to decrease as the concentration of airborne pollen decreases[1,2]. This low efficiency may be due to large distances between conspecifics, low pollen production by individuals, poor pollen dispersal and wind velocity. These conditions are assumed to affect both ovule fertilization and subsequent seed production, negatively[3].

Pinus roxburghii Sargent (Chir pine or Himalayan long-needle pine), a valuable timber-resin tree of the Central–western Himalayan region is also a wind-pollinated species, but little is known about its reproductive biology, particularly pollination ecology. Therefore, the present study was initiated to understand microsporangium protrusion, and pollen liberation and pollen flow in *P. roxburghii* under different weather conditions.

This study was conducted with five representative trees (ten strobili per tree), in the natural populations of *P. roxburghii* at Ashtavakra (900 m amsl) in the Pauri Garhwal district, situated between 29°20′ and 30°15′N lat and 78°10′ and 79°20′E long.

Randomly selected strobili in the crown were examined at 1 h intervals for entire daylengths. Microsporangium dehiscence was recorded by the scoring and removing method, to avoid duplication, with the help of a hand lens (× 20). Prevailing air temperature, relative humidity and wind speed were also recorded each time close to the strobili. Observations were recorded on six different days under different weather conditions: clear sky with heavy dew and little dew; cloudy with no dew and no rain; cloudy with heavy rain and drizzling, and partially cloudy, to decipher exactly the effect of weather factors on microsporangium protrusion.

Pollen concentrations in the atmosphere were observed on jelly-coated microscopic slides, mounted vertically on iron rods and placed perpendicular to the direction of prevailing wind or all around the source tree, viz. north, south, east and west directions. The rods were mounted on the trees at heights corresponding with those of the pollen cones, and the slides were replaced at every 2 h intervals. The number of pollen grains per slide was counted under the binocular microscope on an area of 1 cm^2 (1 cm × 1 cm). Twenty slides were placed around each source tree (five slides per direction). The distance of each slide from the source tree was approximately 5 m. This experiment was performed on three different source trees. Similarly, megasporophylls (stigmas) on ovulate strobili were used to observe pollen concentration and/or the rate of pollen deposition at every 2 h intervals, to decipher the best time of the day for pollination. The stand density of reproductively matured male individuals was 160 trees per hectare. The ovulate strobili were exposed to wind in batches for desired intervals up to three days. They were then removed from the source tree and placed in plastic jars with cotton wadding saturated with formalin-aceto-alcohol, according to Ornduff[4].

The effect of disturbance on pollen concentration was verified by subjecting the physical disturbance to the bloomed bough under undisturbed conditions inside a room by arranging jelly-coated slides at 1 m distance all around, on the same level. The rate of release of pollen grains into the ambient air was also verified by moving a

*For correspondence. (e-mail: vpkhanduri@yahoo.co.in)

fan at various wind speeds to dislodge the pollen grains of a bloomed bough under controlled conditions.

Field observations have revealed that in a majority of strobili, all microsporangia did not dehisce simultaneously, but on an average took 2–7 days (depending on the weather conditions and altitude) to complete the process of dehiscence. Even two lobes of the same pollen sac did not dehisce synchronously.

Table 1 gives the observed times for protrusion and dehiscence of pollen sacs on six different dates in consonance with the prevailing weather conditions. The protrusion occurred due to the elongation of the pollen sacs between 0600 and 1300 h, the duration varying from 2 to 6 h. However, the exact time of initiation of protrusion and duration depends on environmental regimes. Under dew conditions, the initiation occurred within a temperature range of 8.0 to 17°C. During higher humidity conditions, the period of protrusion was relatively more (on 23 February 1998 and 24 February 1999). But on days without dew, protrusion was relatively low (24 February 1998). As plant cells require water during elongation, the cells of pollen-sac filaments would cause mature androecia to protrude when water is available. The incidence of rain between 0600 and 0700 h on 26 February 1998 not only increased/induced protrusion (during 0700 to 1300 h), but also delayed dehiscence. On days with relatively high temperature and humidity, protrusion was observed more due to increased water uptake. Once protruded, the anthers dehisced sooner or later, depending on air temperature and humidity. Moist/wet conditions induced protrusion, while dry conditions favoured dehiscence.

In the climatic chamber (inside a seed germinator), the temperature range of 25 to 28°C causes profuse protrusion within 1 h, but dehiscence was only 2–5% even after 10 h on completion of the anthesis cycle and protrusion of the microsporangia. At room temperature (18.43 ± 0.15°C) with 66.86 ± 2.73% relative humidity, 60–70% dehiscence was observed within 20 h. The pollen cones with protruded androecia, when taken out from the climatic chamber and placed in sunlight conditions (temperature 20–30°C and RH 55–48%), started dehiscence within 15 min and the whole pollen cone dehisced just within 2 h. Thus, dehiscence occurs due to desiccation of pollen-sac walls, which requires ambient temperature above 20°C and RH below 60%.

The pollen grains were found liberated inside a room when the pollen cones were gently shaken, manually. Further, increased disturbance to the bloomed bough was caused by moving a fan at wind speeds of various degrees, i.e. 0.5 to 3.5 m/s. The results have shown that 76 pollen grains per cm^2 were observed with 0.5 m/s wind speed, which is 6.19% of the total pollen grains trapped at various degrees of wind speed. On the other hand, the highest pollen frequency (306 grains/cm^2, i.e. 24.94% of the total pollen grains) was observed at wind speed of 3.0 m/s (Figure 1).

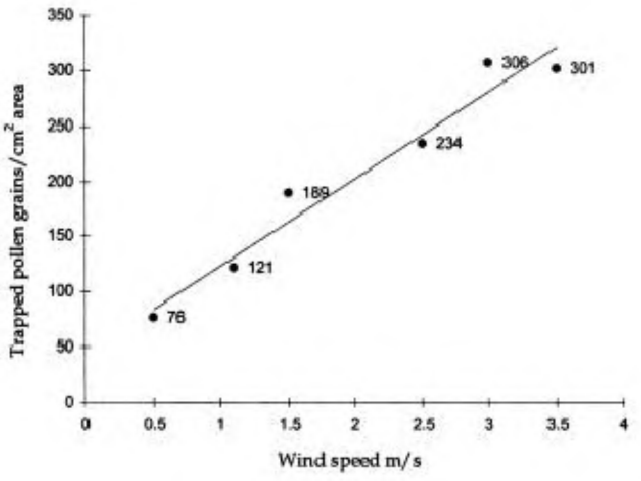

Figure 1. Variation in rate of artificial pollen release under various wind speeds.

Receptivity of megasporophylls varied from 72 to 120 h (i.e. 3–5 days) as indicated by the scales of ovulate strobili. However, longer receptivity period (i.e. 144 h) was indicated occasionally by the ovulate strobili which were isolated initially but opened at different intervals, while observing the pollen loads. The observed differences in the duration of receptivity might be due to the micro-climate of bagged strobili, which is supposed to be more humid.

The census of airborne pollen grains in a single day on two-hourly basis has shown that the maximum concentration of pollen grains in air was 109 ± 7.84 and 193 ± 12.69 grains/cm^2 (on day 1), 134 ± 7.43 and 101 ± 3.24 grains/cm^2 (on day 2), and 104 ± 3.18 and 119 ± 6.41 grains/cm^2 (on day 3) between 1200 and 1400 h and 1400 and 1600 h respectively, during which peak pollen deposition on megasporophylls was also noticed. This was 26 ± 1.32 and 31 ± 2.10 grains/cm^2 (on day 1), 36 ± 1.53 and 29 ± 1.74 grains/cm^2 (on day 2) and 22 ± 2.37 and 27 ± 1.12 grains/cm^2 (on day 3) respectively (Table 2). The higher rates of pollen deposition onto the megasporophylls were verified by observing the time of peak periodicity of pollen grains in air. It is therefore clear that in *P. roxburghii* the deposition of pollen grains on megasporophylls is maximum between 1200 and 1600 h during the day.

Climatic factors influence the anthesis, microsporangia protrusion, and dehiscence, and ultimately pollen release, in *P. roxburghii*. The air temperature must exceed up to a level of 20 to 30°C and relative air humidity must remain below 85% to start the process of anthesis. However, the presence or absence of light did not affect the anthesis[5]. High percentage of relative air humidity favours microsporangia protrusion. Wet conditions caused by dew and/or rain not only delayed the dehiscence but also prolonged it, as was the case on 26 February 1998 due to inci-

Table 1. Observed times of protrusion and dehiscence of pollen sacs and associated weather conditions

Variable	Time of the day (h) 0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	1800	PMP	Dew/ rain	NS
Temperature	8.0	9.1	10.3	11.5	14.0	16.4	18.6	19.1	19.0	16.2	15.4	13.0	11.3	0600	Heavy	Clear
RH	86	83	80	75	72	67	61	57	56	59	64	68	73	to	dew	sky
WS	0.05	0.08	0.3	0.5	0.8	0.9	1.1	1.2	2.3	2.5	1.6	1.1	0.6	1100		
DM	–	–	1.70	3.82 ± 0.49	6.52 ± 0.63	12.34 ± 0.72	17.39 ± 0.76	20.43 ± 0.69	18.14 ± 0.89	11.34 ± 0.63	5.53 ± 0.39	3.24 ± 0.29	1.41 ± 0.21			
Temperature	8.4	8.9	10.2	11.3	12.2	14.1	16.5	16.8	15.4	15.0	12.4	10.3	10.0	0600	No dew	Cloudy
RH	82	81	82	80	81	79	76	78	77	80	81	80	83	to	and	
WS	0.1	0.3	0.5	0.6	0.8	0.6	1.0	0.8	2.0	2.6	1.7	1.0	0.6	0800	no rain	
DM	–	–	–	–	–	1.46 ± 0.23	3.82 ± 0.32	4.62 ± 0.19	4.12 ± 0.26	3.42 ± 0.41	1.41 ± 0.12	–	–			
Temperature	8.5	8.9	10.2	11.5	13.0	14.3	15.7	17.8	17.4	15.3	14.5	12.3	11.7	0600	Dew	Partially
RH	83	81	80	80	77	75	72	66	65	69	74	77	81	to		cloudy
WS	0.08	0.1	0.3	0.1	0.4	0.6	1.1	1.7	1.9	1.2	0.7	0.6	0.2	0900		between
DM	–	–	–	–	4.50 ± 0.37	7.63 ± 0.42	10.42 ± 0.53	12.43 ± 0.32	15.56 ± 0.57	13.14 ± 0.63	11.60 ± 0.43	7.36 ± 0.21	–			0600 and 1200 h
Temperature	7.0	7.3	8.1	9.4	12.3	14.1	16.6	12.6	10.5	9.2	11.4	10.6	9.7	0700	Dew,	Cloudy
RH	87	88	86	84	83	81	80	84	92	86	85	82	81	to	drizzling	
WS	0.04	0.1	0.3	0.2	0.4	0.6	2.5	6.7	–	1.2	1.0	0.7	0.5	1300	between	
DM	–	–	–	–	–	–	3.45 ± 0.13	1.24 ± 0.17	–	–	–	–	–		0600 to 0700 h and heavy rain between 1400 and 1500 h	
Temperature	9.4	10.0	11.3	12.2	13.6	15.8	20.6	20.8	20.4	18.0	16.7	14.5	13.2	0600	Little dew	Clear sky
RH	81	80	76	74	71	66	59	56	57	59	63	67	72	to		
WS	0.06	0.1	0.3	0.2	0.2	0.5	0.8	2.3	2.0	2.7	3.5	1.6	1.3	0900		
DM	–	–	1.40 ± 0.13	3.20 ± 0.27	4.12 ± 0.31	8.34 ± 0.21	14.12 ± 0.41	16.41 ± 1.05	17.00 ± 1.10	10.34 ± 0.73	9.43 ± 0.51	4.24 ± 0.27	2.15 ± 0.34			
Temperature	9.6	10.1	11.2	11.8	13.9	15.3	18.2	17.4	15.2	14.2	14.0	13.5	12.9	0600	Dew,	Partially
RH	80	81	78	74	72	70	68	73	86	90	90	84	80	to	drizzling	cloudy
WS	0.1	0.08	0.2	0.4	0.7	0.9	3.4	3.8	2.1	1.6	1.0	0.7	0.6	1000	between	between
DM	–	–	2.41 ± 0.23	3.17 ± 0.26	3.78 ± 0.18	5.34 ± 0.43	9.47 ± 0.59	12.56 ± 0.70	–	–	–	–	–		1400 and 1600 h	0600 and 1200 h

Table 2. Temporal variation in atmospheric pollen concentration in natural and artificial trappers (20 to 26 February 1999)

	Stigmatic pollen load (natural trapper)			Frequency on jelly-coated slide (artificial trapper)			
Time (h)	Day-1 Grains deposited/ megasporophyll	Day-2 Grains deposited/ megasporophyll	Day-3 Grains deposited/ megasporophyll	Day-1 No. of pollen grains deposited/cm^2	Day-2 No. of pollen grains deposited/cm^2	Day-3 No. of pollen grains deposited/cm^2	Average wind speed (m/s)
0600	–	–	–	–	22 ± 1.43	15 ± 1.12	0.6 ± 0.02
0800	07 ± 0.19	05 ± 0.07	09 ± 0.13	21 ± 1.73	14 ± 1.10	19 ± 0.93	0.27 ± 0.09
1000	12 ± 0.43	14 ± 0.29	15 ± 0.34	46 ± 1.92	32 ± 1.69	54 ± 1.54	0.53 ± 0.09
1200	19 ± 1.12	22 ± 0.87	26 ± 0.68	89 ± 3.14	70 ± 2.13	104 ± 3.18	1.45 ± 0.09
1400	26 ± 1.32	36 ± 1.53	22 ± 2.37	109 ± 7.84	134 ± 7.43	119 ± 6.41	2.27 ± 0.15
1600	31 ± 2.10	29 ± 1.74	27 ± 1.12	193 ± 12.69	101 ± 3.24	97 ± 2.34	2.40 ± 0.38
1800	21 ± 1.04	24 ± 0.68	20 ± 0.71	54 ± 1.71	42 ± 2.17	48 ± 2.10	0.36 ± 0.11
2000	09 ± 0.13	10 ± 0.51	07 ± 0.11	40 ± 1.21	21 ± 1.31	32 ± 1.72	0.25 ± 0.08

dence of rain between 0600 and 0700 h, when the dehiscence started five hours late after protrusion (Table 1).

The syngenesious nature and lateral position of the line of dehiscence facilitate the abutting pollen sacs to hold loosely the pollen, until liberated into the ambient air by some kind of disturbance. In nature, wind is the major factor causing continuous disturbance to pollen cones, which triggers pollen release. A strong relationship was noticed between the number of captured pollen grains and the wind velocity (as is evident from Figure 1). On a day when the lowest wind velocity was observed before noon, lesser number of pollen grains were captured, after which when highest wind velocity was observed in the afternoon, the largest number of pollen grains were liberated. Similar results have been obtained by various researchers in *P. sylvestris*[6,7], *Cedrus deodara*[8], *Xanthium*[9], *Emblica* and *Cicca*[10]. Obviously, pollen that appeared in air are consequent upon the onset of superadiabatic (turbulent) conditions.

Based on our knowledge of the aerodynamics of particle transport and capture, it has now been established that the extent of dispersal is controlled by an interaction of the terminal velocity of the pollen and wind velocity, and that the actual concentration of pollen in the atmosphere at any point is influenced by disturbance in the atmosphere[11,12]. In *P. roxburghii*, there occurs a regular daytime maximum and nightly minimum pollen concentration. The time between 1200 and 1600 h was considered as the best time for pollination during the day. According to Ingold[13], 'the wind pollinated plants shed pollen almost entirely during daytime and pollen are set free under conditions suitable for effective dispersal', which is also, supported by the present results. Tauber[14–16] emphasized that pollen grains fall because the gravity reaches a terminal velocity between 1 and 10 cm/s. Jacobson and Bradshaw[17] described that in the absence of wind, downward movement of pollen grains within the gravitational field is called the gravity component of pollen movement or vertical dryfall of pollen from local vegetation.

The aerial pollen concentrations are dependent on pollen-shedding, take-up of pollen grains into the air and their transport by wind and thermal convection. Rain causes disturbance to this series of events. These phases are affected differently by weather conditions. The opening of anthers or pollen release is caused by drying. On the other hand, in high relative humidity pollen concentrations are clearly lower. The rupture of anthers might be affected by enzymatic breakdown[18], which means that this phase might also be temperature-dependent.

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Plant regeneration via organogenesis from shoot base-derived callus of *Arachis stenosperma* and *A. villosa*

G. Vijaya Laxmi and C. C. Giri*

Centre for Plant Molecular Biology, Department of Genetics, Osmania University, Hyderabad 500 007, India

Plant regeneration via organogenesis was obtained from shoot base-derived callus cultures of wild *Arachis* species, viz. *Arachis stenosperma* and *Arachis villosa*. The callus was induced from the pulse-treated (5000 ppm IBA for five minutes) shoot bases on MS medium supplemented with 11.42 µM IAA. Green spots (meristemoids) and purple-coloured leafy shoots were observed from the shoot base-derived callus within 2–4 weeks of culture on a medium containing half strength MS nutrients and B5 vitamins supplemented with 22.20 µM BA, 2.85 µM IAA and 1.0 µM TDZ. Shoot regeneration frequency up to 75% and 68% was observed in *A. stenosperma* and *A. villosa* respectively. Anatomical study revealed the organogenic pathway of regeneration from callus cultures. The regenerated shoots of *A. stenosperma* were rooted with a frequency of 52% on media supplemented with 16.12 µM NAA and 2.32 µM kinetin. In *A. villosa*, optimum response of 25% rooting was observed on media supplemented with 11.4 µM of IAA and 2.32 µM kinetin. The rooted plantlets were transferred to soil: vermiculite (1 : 1) mixture for two weeks fortified with ½ MS nutrients and finally transferred to soil in pots with 75% survival rate. The regenerated plantlets of both the wild species flowered and set seed normally.

THE grain legumes are important group of crops with major source of dietary protein and oil. The wild genotypes of *Arachis* are valuable sources of resistant genes against several pests, pathogens besides high oil and protein content[1–4]. The groundnut (*Arachis hypogaea* L.), a major oil seed crop rich in protein, makes a substantial contribution to human nutrition. Due to lack of resistance to biotic and abiotic stresses in cultivated groundnut, the productivity remained low despite large acreage under cultivation.

The multiplication and maintenance of wild *Arachis* germplasm is very labour-intensive and involves specific protocols because many accessions are grown mostly under greenhouse/glasshouse conditions. For instance, the field-grown wild plants are uprooted and the soil has to be sifted to harvest the seeds[5]. Therefore, there is a limited supply of wild germplasm from the gene bank and it becomes difficult to maintain wild species of *Arachis* for its use in breeding programme.

Plant regeneration until the recent past in cultivated and in wild groundnut has been achieved either directly via organogenesis or indirectly through somatic embryogenesis[6–12]. However, the reports on plant regeneration with intervening callus phase are few in cultivated genotypes and less in wild *Arachis*[13–16]. The standardization of *in vitro* plant regeneration protocols with intervening callus phase would certainly help in the mass scale propagation of the wild species and also facilitate germplasm conservation *in vitro*[17]. In addition, the protocol can also be exploited for generating new genetic variability in groundnut by somatic hybridization through protoplast fusion as has been demonstrated in other legumes[18].

Further, this is an attempt to study the effect of different plant growth regulators on morphogenetic response of shoot base-derived callus. The present study deals with organogenic regeneration from shoot base-derived callus of *A. stenosperma* and *A. villosa*. *A. stenosperma* is resistant to late leaf spot and *A. villosa* confers drought tolerance and shows resistance to tikka disease, insect pests and has high oil content[1,19].

The seeds of wild species, i.e. *A. stenosperma* and *A. villosa*, ($2n = 2x = 20$) were collected from Gene Bank of ICRISAT, Patancheru, Hyderabad, India. The seeds were surface sterilized using 0.1% (w/v) mercuric chloride solution for 8 min followed by 5–6 times thorough washing with sterile distilled water. The seeds were germinated on filter paper boat on liquid half strength MS[20] media with B5[21] vitamins in culture tubes. The experiments were conducted in our lab for *in vitro* induction of rooting in wild species using a range of IBA (0–6000 ppm) solution for different time durations (1–10 min). Preliminary experiments revealed the shoots pulse-treated with 5000 ppm for 5 min were found to induce maximum callusing within 7–10 days on transfer to MS media supplemented with 11.42 µM IAA or 10.75 µM NAA compared to other concentrations of these auxins tested. Hence, in the present study, ten-day-old seedling shoots were excised and the cut ends were given pulse treatment with 5000 ppm solution of IBA for 5 min. The pulse-treated shoots were

*For correspondence. (e-mail: giriccin@yahoo.co.in)