TEMPORAL AND SPATIAL VARIATION IN AIRBORNE POLLEN AND QUALITY OF THE SEED CROP IN A NORWAY SPRUCE SEED ORCHARD

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ABSTRACT

Temporal and spatial variation in airborne pollen, and in the quality of the seed produced, were studied in a Norway spruce (*Picea abies*) seed orchard, located in southern Finland (62° 13' N, 25° 24' E), consisting of 67 clones from northern Finland (64°–67° N). Data for the study were collected in 1995, and consisted of the results of pollen sampling, cone and seed measurements, and isozyme analysis.

Duration of anthesis during 1995 was 5 days. The amount of airborne pollen increased during the first four days, and then decreased rapidly. Diurnal variation was high; the lowest amounts of pollen being measured at night and in early morning when, in general, air humidity was high and wind speed low. During the first two days of anthesis, pollen densities inside and outside the orchard were approximately the same, but from the third day onwards the densities in the orchard were higher. On the third day, the highest densities were measured on the southern slope, but one day later in the northern part of the orchard, indicating phenological differences in pollen shedding. In addition to phenology, spatial variation was affected by the wind; the highest pollen densities were measured on the downwind side of the orchard. The spatial variation in the amount of airborne pollen correlated significantly with pollen contamination: contamination was high (0.80) on the eastern, upwind side of the orchard, and much lower (0.51–0.57) in the middle and northwest part. The estimated rate of pollen contamination for the whole seed orchard was 0.71, while the rate of self-fertilisation was 0.06 with no significant spatial variation. In addition, spatial variation was found in cone production and seed characteristics.

Keywords: *Picea abies*, pollination, background pollination, pollen contamination, self-fertilisation, inbreeding, reproductive synchronisation

INTRODUCTION

In natural populations of wind-pollinated conifers, gene flow via pollen and seed is efficient, resulting in conifer populations to exhibit high level of genetic variation within populations, large effective population size, but small variation among populations (GOVINDARAJU 1989; MUONA 1990; ADAMS 1992; MÜLLER-STARCK *et al.* 1992). The amount and distance of pollen distribution have been studied for a long time (WRIGHT 1953; LANNER 1966; KOSKI 1970; SORENSEN 1972), and it has been shown that considerable amounts of viable pollen can fly from population to another, and even over long distances (WHEELER *et al.* 1993; LINDGREN *et al.* 1995, LINDGREN & LINDGREN 1997).

In seed orchards, gene flow from outside sources, i.e. pollen contamination, has three different kinds of effect. Firstly, it will raise or at least maintain the genetic diversity of the seed produced (SAVOLAINEN & KÄRKKÄINEN 1992; NIKKANEN & RUOTSALAINEN 2000). Secondly, a high level of pollen contamination, observed in many wind-pollinated seed orchards (EL-KASSABY *et al.* 1989, HARJU & MUONA 1989; PAKKANEN & PULKKINEN 1991; WANG *et al.* 1991; YAZDANI & LINDGREN 1991; PAKKANEN *et al.* 2000), significantly reduces the genetic gain that can be obtained from seed orchards (LOWE & WHEELER 1993). Thirdly, gene flow may reduce the adaptability of seedlings originating in seed orchards that are established outside the geographic origin of their clones (NIKKANEN 1982; LOWE & WHEELER 1993; RUOTSALAINEN & NIKKANEN 1998). As a consequence, pollen contamination from non-selected natural forests is considered a major problem in many conifer seed orchards (DI-GIOVANNI & KEVAN 1991; LINDGREN 1991; SAVOLAINEN 1991; BUCHERT 1992; DI-GIOVANNI & JOYCE 1992; WHEELER & JECH 1992).

The procedures used to estimate pollen distribution and contamination in seed orchards can be divided into two categories, namely trapping of airborne pollen (DI-

GIOVANNI & KEVAN 1991; DI-GIOVANNI & JOYCE 1992; WHEELER *et al.* 1993), and paternity analysis of seeds by means of isozyme or DNA-marker techniques (BUCHERT 1992; WHEELER & JECH 1992; FRIEDMAN & NEALE 1993; LOWE & WHEELER 1993; WHEELER *et al.* 1993). There are a range of pollen trapping techniques that are used for different purposes (SARVAS 1955; SORENSEN 1972; SOLOMON *et al.* 1980; DI-GIOVANNI & JOYCE 1992). The type of recording pollen sampler, developed and described by SARVAS (1962; 1968), can be used specifically for studying temporal variation in airborne pollen, and the rotorod type of sampler, developed to measure the densities of different particles in the air (EDMONDS 1972), can be used for studying spatial variation in airborne pollen. In conifers, paternity analyses have frequently been performed on the basis of multilocus allozyme markers (SHAW *et al.* 1981; SMITH & ADAMS 1983; FRIEDMAN & ADAMS 1985; MUONA *et al.* 1987), but in recent years also DNA markers, such as RAPD fragments (LU *et al.* 1995; KHASA & DANCIK 1996; SZMIDT *et al.* 1996), chloroplast microsatellites (ZIEGENHAGEN *et al.* 1998; PLOMION *et al.* 2001) and (polymorphic) EST-PCR markers (SCHUBERT *et al.* 2001) have been developed for this purpose.

The aim of this study was to investigate temporal and spatial variation in airborne pollen in a Norway spruce (*Picea abies* (L.) Karst.) seed orchard and its immediate surroundings, and to estimate pollen contamination and self-fertilisation in different parts of the orchard. The hypothesis of the study was that the rate of pollen contamination might be affected by temporal and spatial variation in airborne pollen. Both pollen trapping and paternity analysis were applied in the study. An additional aim of the study was to estimate spatial variation in some of the quality characteristics of the seed produced.

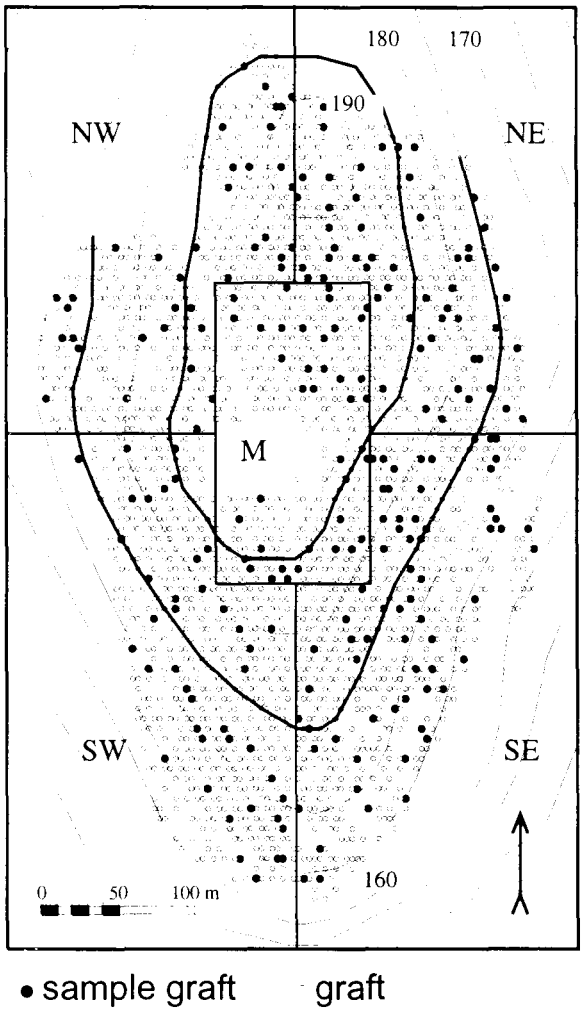

Figure 1 The Heinämäki seed orchard. Sample grafts for isozyme analysis, as well as the borderlines of altitude zones, and sections of the orchard, are marked on the map.

MATERIAL AND METHODS

The seed orchard

Variation in the amount of airborne pollen and in the quality of the seed produced were studied in Norway spruce seed orchard no. 170, Heinämäki, established in 1968 at Korpilahti, southern Finland (62° 13' N, 25° 24' E). The seed orchard consists of 67 clones originating from latitudes 64°–67° N in northern Finland.

The seed orchard is 13.2 ha in size, and is located on a hill (160–190 m asl) sloping gently to the south and steeply to the east and west (Fig. 1). The grafts were planted in the orchard using a clonal-row design with ramets of each clone in two or more rows. The initial spacing of the grafts was 3.5 × 6.5 m, the ramets of the same clone being located 6.5 m from each other. The seed orchard has been thinned systematically by removing every third graft. The average height of the grafts was 10.0 m. For more details about the growth and flowering of the grafts, as well as management of the seed orchard, see NIKKANEN and RUOTSALAINEN (2000), NIKKANEN (2001), and NIKKANEN (2002). Norway spruce is the predominant tree species in the region of the orchard.

The topography of the seed orchard and its surroundings and the position of the grafts and pollen samplers were determined in 1993 by means of a tachymeter (Nikon A20) and a field computer (Geonic 1000). The equipment was used to create a three-dimensional coordinate system covering the study area.

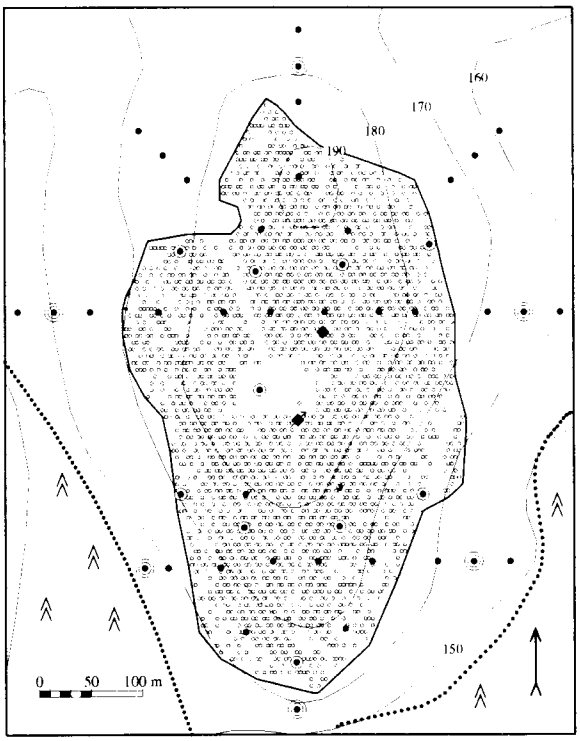

Figure 2. The Heinämäki seed orchard and its immediate surroundings. Locations of the pollen samplers and the weather station, and the borderlines of the orchard and the spruce forest, are marked on the map.

Climatic observations

The weather data for the study period in 1995 were obtained from the Jyväskylä weather station of the Finnish Meteorological Institute, located 25 km northeast of the seed orchard, and from a weather station (Datataker 610) inside the orchard (Fig. 2). The weather data from Jyväskylä weather station consisted of daily mean temperatures, effective temperature sum (d.d., >+5 °C), cloudiness, and precipitation. The data from the within orchard weather station consisted of continuous temperature, illuminance, humidity, precipitation, and wind speed and direction during the flowering period (Table 1). Wind speed and direction was measured at the height of 9.4 m.

Pollen sampling

Temporal variation in airborne pollen was measured by means of a recording pollen sampler (SARVAS 1968), located in the centre of the seed orchard at the height of 9.4 m (Fig. 2). Spatial variation in airborne pollen was studied using a rotorod type of sampler (EDMONDS 1972). A total of 70 samplers were situated on 48 masts, 1–3 samplers on each mast; 48 samplers were at the height of 4.5 m, 16 at the height of 9.0 m, and 6 at the height of 13.0 m (Fig. 2). A total of 37 of the samplers were located in the seed orchard and 33 outside it. A total of 24 ten-minute sampling periods were achieved during the seven-day period from 28 May to 3 June, 1995 (Table 1).

The rotorod sampler consisted of a pair of rods, 92 mm apart, rotated at a constant speed by a means of an electric motor with an average rotation speed of 2100 rpm (it varied from 1840 to 2410 rpm depending on the distance between the sampler and its battery). With a sampling period of 10 minutes and 8 observation views of 0.7 mm^2 in each rod, the average volume of air swept was 0.067 m^3. Pollen grains were trapped on a thin film of Vaseline in the collector rods. The number of pollen grains caught was counted using a microscope (Wild M 20) and special reading equipment.

Cone and seed sampling

Cones from 490 grafts from 66 clones were sampled for the target of collection, and cones were collected during September 1995. The cone and seed crops were determined separately for each graft. In addition to the number and volume of cones, the number of damaged cones was also counted. After extracting the seeds, the weight of the seed crop, 1000-seed weight, and the number of seeds per cone were determined for each graft. In addition, the percentage of full seed was determined by x-ray analysis (SIMAK 1980; NUMMINEN & HÄGGMAN 1987) using 400 seeds from one clone, each of which consisted on the average of 6 grafts.

Statistical analyses

Temporal variation in the amount of airborne pollen was analysed by non-linear regression analysis. Statistical differences in cone and seed characteristics among the clones, and between the zones and sections of the orchard, were studied by analysis of variance and the Tukey post-hoc test using the GLM General Factorial procedure. The strength of a linear association between different variables was assessed by Pearson correlation coefficients. The analyses were performed by SPSS® 10.0 statistical software (SPSS Inc. 1999).

Spatial analysis of airborne pollen

Spatial variation in airborne pollen was described by means of density maps. The pollen density values at

Table 1. Weather conditions in 1995 in the Heinämäki seed orchard during rotorod sampling (10 min periods) of airborne pollen, measured using a Datataker 610 weather station.

Sampling date and time		Temperature °C	Luminance $W \cdot m^{-2}$	Humidity %	Wind	
					speed $m \cdot s^{-1}$	direction °
May 28	13	23.1	757	37	2.4	256
May 29	10	22.5	585	50	1.0	158
	13	26.5	294	42	2.3	162
	16	26.5	625	44	3.3	155
May 30	10	23.9	578	49	1.2	228
	13	27.0	725	40	2.8	213
	16	26.7	668	40	1.2	201
	19	26.6	266	39	1.7	274
May 31	7	17.2	269	68	2.0	135
	10	21.9	610	49	1.8	226
	13	26.1	759	36	0.5	55
	16	25.9	618	30	2.1	274
June 1	1	16.7	18	72	1.0	38
	7	15.3	268	81	1.7	75
	10	17.9	599	67	2.4	58
	13	22.7	742	53	2.2	74
	16	25.0	618	48	2.3	89
	19	23.1	122	49	2.4	137
	22	18.9	22	76	0.8	94
June 2	10	23.7	590	65	2.4	119
	13	28.0	798	50	2.3	93
	16	26.9	175	42	2.3	158
June 3	10	27.3	607	47	0.5	121
	13	28.9	769	42	1.5	116

each sampling time were interpolated for the 20 × 20 meter grid covering the whole study area. Interpolation was performed by applying ordinary kriging (CRESSIE 1993, p. 120). The spatial correlation of pollen density was expressed as a symmetrical spherical variogram model (PEBESMA 1999). The parameters of the variogram model were estimated using pooled data from all the samplings, and the same estimates of the parameters were then used in each sampling. Kriging was computed on the logarithmic scale, and the interpolated logarithmic values were transformed into the original scale for map drawing. Kriging was performed by Gstat 2.2.1 software (PEBESMA 1999).

Estimation of pollen contamination and self-fertilisation

A total of 238 grafts from 52 clones were used as material for the pollen contamination and inbreeding analyses. The grafts were chosen to cover all the zones and sections of the orchard, as well as all the seed-producing clones. A total of 2838 seeds were analysed. The multilocus genotypes of the embryos and haploid megagametophytes were assessed at the following 11 allozyme loci: acid phosphatase (E.C.3.1.3.2.), aconitase (E.C.4.2.1.3.), diaphorase (E.C.1.6.4.3.), fluorescent esterase (E.C.3.1.1.1.), glutamate dehydrogenase (E.C.1.4.1.2.), two loci of glutamate oxaloacetic transaminase (E.C.2.6.1.1.), two loci of leucine amino peptidase (E.C.3.4.11.1.), malate dehydrogenase (E.C.1.1.1.37.) and phosphoglucose isomerase (E.C.-5.3.1.9.). For details of the technique used and the formal genetics of these loci, see MUONA *et al.* (1987).

The multilocus genotypes of pollen gametes were deduced by comparing the allozyme patterns in the megagametophytes with those in the corresponding embryos. Pollen genotypes that could not have been produced by any of the seed orchard clones were regarded as detected contamination (*b*). Because part of the contaminating pollen could not be distinguished

from pollen produced by the orchard clones, the detected contamination (b) had to be adjusted by the detection probability (d) of alien pollen in order to obtain the estimate of pollen contamination rate (m) as $m = b / d$ (SMITH & ADAMS 1983). The single locus embryo gene frequencies of 400 seeds, collected in 1992 from 99 trees in a Norway spruce stand close to the Heinämäki seed orchard, were used to obtain an estimate of the detection probability. The formula used to estimate the variance of contamination is given by FRIEDMAN and ADAMS (1985). The multilocus method of SHAW *et al.* (1981) was used to estimate the proportions of outcrossing (t), and selfing ($= 1-t$) rates. Pollen gametes not matching the mother tree genotype were regarded as outcrossings. The estimated outcrossing rate was obtained by adjusting the detected outcrossing rate by the probability to detect the selfings, which was estimated by means of the gene frequencies of the seed orchard. The pollen contamination and self-fertilisation rates were estimated for the whole seed orchard, and separately for the zones and sections of the seed orchard. Differences in pollen contamination and self-fertilisation between the zones and sections were analysed using the Pearson χ^2-test.

RESULTS

Temporal and spatial variation in airborne pollen

Duration of anthesis during 1995 in the Norway spruce seed orchard was 5 days (Fig. 4). During this period (May 29 to June 2) the daily mean temperatures varied from 19.5 to 22.0 °C, with an average of 21.2 °C, and the heat-sum accumulated from 85 to 166 d.d.. The average number of sunny hours per day was 13.2, varying from 10.1 to 15.9, and slight rain occurred only on June 2. During the period wind direction, as well as wind speed, varied from day to day (Table 1). The maximum wind speed varied from 3 to 4 $m \cdot s^{-1}$, except on May 29 when it was 4.7 $m \cdot s^{-1}$. The weather conditions during the rotorod sampling are shown in Table 1.

The amount of pollen in the air was highest on the fourth day of anthesis; the average pollen density at 4 p.m. at the height of 9 m in the orchard was 4000 pollen grains per m^3 of air (Fig. 3). Pollen densities, measured on the same masts, were 3.5 % higher at the height of 4.5 m than at the height of 9 m, and were significantly correlated ($r = 0.96$, $p < 0.0001$). The amount of pollen measured by the two different types of pollen sampler was also highly significantly correlated ($r = 0.94$, $p < 0.0001$).

Diurnal variation in the amount of airborne pollen was high; at night and in the early morning much less pollen was in the air than during daytime (Fig. 3). The logarithm of the amount of pollen was expressed as a function of heat-sum, humidity and wind speed. The coefficients of the model were estimated using restricted non-linear regression analysis, the restriction being that the values of the two polynomials are equal at the temperature sum of 143 d.d., when the amount of pollen reached its maximum value. The estimated model was

$$\ln(pollen + 0.1) = I_{143} * (-4.82 + 0.07 * D + 0.10 * (D/100)^3) + (1 - I_{143}) * (18.00 - 0.09 * D + 0.11 * (D/100)^3) - 0.05 * H + 1.40 * \ln(W + 0.5)$$

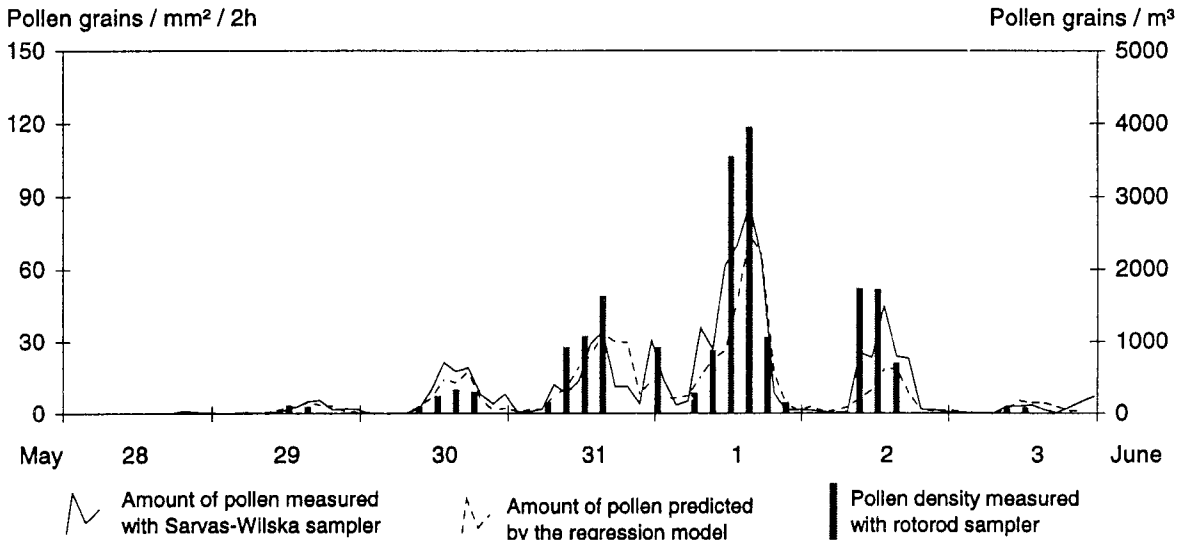

Figure 3. Variation in the amount of airborne pollen caught with different pollen samplers, and the prediction of the variation during the flowering period in 1995 in the Heinämäki seed orchard.

Table 2. Number of sample grafts, cones per graft and some seed characteristics in different altitude zones (a) and in different sections (b) of the Heinämäki seed orchard, and significance for differences in ANOVA. The means marked with different letters (m, n) differ significantly from each other, $p < 0.05$ in the Tukey post-hoc test.

a

Altitude zone asl	Number of grafts	Number of cones per graft		Number of seed per graft		1000-seed weight (g)		Percentaqge of full seed	
<175 m	171	126	m n	17.8	m	4.6	m	38	m
175-184 m	154	118	m	18.1	m	4.8	m n	42	m
≥185 m	159	185	n	30.9	n	5.0	n	49	n
Total	484	143		22.3		4.8		43	
F		3.482		18.489		5.030		15.583	
p		0.032		0.000		0.008		0.000	

b

Section of the seed orchard	Number of grafts	Number of cones per graft	Number of seed per cone		1000-seed weight (g)		Percentaqge of full seed	
NW	52	194	25.4	m n	4.5	m	49	n
NE	128	138	23.0	m n	4.8	m n	43	m n
SE	142	114	19.5	m	4.6	m	39	m
SW	94	125	17.4	m	4.8	m n	43	m n
Middle	68	198	29.8	n	5.3	n	47	n
Total	484	143	22.3		4.8		43	
F		2.024	3.834		3.811		4.478	
p		0.090	0.005		0.005		0.002	

ences started to increase during the later part of anthesis (Fig. 4). In the afternoon of the third day the highest pollen densities were measured on the southern slope, while on the fourth and fifth days they occurred in the northern part of the orchard. The accumulated amount of pollen during anthesis was almost two times higher in the western and middle sections (NW, SW and M) than in the eastern sections (NE and SE) of the orchard.

On the first two days of anthesis the average pollen densities inside and outside the orchard were about the same, however, the average density inside the orchard was 3 and 1.5 times higher than outside for the third, and the fourth and fifth days, respectively (Fig. 5).

Variation in cone and seed production

The average number of cones produced per graft was 143, varying from 0 to 2400. There were large and significant differences in the studied cone and seed characteristics among the clones. The clonal mean of cones per graft varied from 0 (6 clones) to 1150, and the mean of seeds per cone ranged from 3 to 84. The clonal mean of the proportion of full seed varied from 13 to 70 %. The number of seeds per cone and the percentage of full seed correlated significantly (r = 0.528, $p < 0.0001$).

The number of cones per graft, as well as the number of seeds per cone, 1000-seed weight, and the percentage of full seed differed in most cases significantly between the altitude zones and the sections of the orchard (Table 2). The highest values in all cone and seed characteristics occurred in the uppermost zone and middle section of the orchard. High correlation (r = 0.88, p = 0.051) was found between the section means of full seed and the accumulated amount of pollen (Table 3).

Pollen contamination and self-fertilisation

The proportion of detected alien pollen was 0.079 for the whole seed orchard. When the detection probability was 0.11 the estimated pollen contamination for the orchard was 0.71, varying from 0.60 to 0.87 for the different altitude zones, and from 0.51 to 0.80 for the different sections of the orchard (Table 4). The differences in the rate of pollen contamination were signifi-

Table 3. The Pearson correlation coefficients (significance in parentheses) between different characteristics of pollination and seed crop in different sections of the Heinämäki seed orchard.

Characteristic	Accumulated pollen amount	Number of seeds per cone	Percentage of full seed	Self-fertilisation
Number of seed per cone	0.58 (0.307)			
Percentage of full seed	0.88 (0.051)	0.73 (0.163)		
Self-fertilisation	-0.24 (0.702)	-0.73 (0.166)	-0.17 (0.781)	
Pollen contamination	-0.89 (0.044)	-0.85 (0.069)	-0.86 (0.063)	0.50 (0.394)

Table 4. Number of analysed seeds, detected and estimated pollen contamination and self fertilisation rates and their standard deviations in their altitudinal zones (a) and in different sections (b) of the Heinämäki seed orchard, and significance for differences in the Pearson χ^2-test.

a

Altitude zone asl.	Number of seeds	Detected contamination ± (sd)	Estimated contamination ± (sd)	Estimated selfing ± (sd)
< 175 m	953	0.095 (0.010)	0.87 (0.09)	0.075 (0.009)
175–184 m	1007	0.067 (0.008)	0.60 (0.07)	0.061 (0.008)
≥ 185 m	878	0.074 (0.009)	0.67 (0.08)	0.057 (0.008)
Total	2838	0.079 (0.005)	0.71 (0.05)	0.064 (0.005)
F		6.032	174	2.675
p		0.049	0.000	0.263

b

Section of the seed orchard	Numbers of seeds	Detected contamination ± (sd)	Estimated contamination ± (sd)	Estimated selfing ± (sd)
NW	427	0.063 (0.012)	0.57 (0.11)	0.073 (0.013)
NE	623	0.088 (0.011)	0.80 (0.10)	0.063 (0.010)
SE	834	0.088 (0.010)	0.79 (0.09)	0.066 (0.009)
SW	527	0.084 (0.012)	0.76 (0.11)	0.072 (0.011)
Middle	427	0.056 (0.012)	0.51 (0.10)	0.044 (0.010)
Total	2838	0.079 (0.005)	0.71 (0.05)	0.064 (0.005)
F		6.250	181	3.882
p		0.181	0.000	0.422

cant both between the zones and the sections. The highest contamination was estimated for the lowermost altitude zone, and the lowest for the middle section of the orchard (Table 4). Significant negative correlation ($r = -0.89$, $p = 0.044$) was found between the contamination and the accumulated amount of airborne pollen (Table 3). The rate of estimated self-fertilisation was 0.06 for the whole seed orchard (Table 4). The differences between the altitude zones and different sections were not significant.

DISCUSSION

In the Heinämäki seed orchard flowering in 1995 occurred some days later (May 29 to June 2) than the average (NIKKANEN 2001). Weather during the flowering period was exceptionally warm, the mean temperature being 21.0 °C when it is normally 5 to 10 degrees less (NIKKANEN 2001). As a result, the flowering period was compact and lasted 5 days only. In years with colder and more cloudy and rainy weather, the duration

of flowering is longer (average duration over seven different years is 7 days, varying from 5 to 10 days (NIKKANEN 2001)).

Temporal variation in airborne pollen was analysed by non-linear regression analysis, and the model obtained employed heat-sum, air humidity and wind speed to give a rather good fit for predicting the variation in the amount of pollen (Fig. 3). The other variables measured, temperature and illuminance, did not improve the power of the model. The model has not been tested in other years or other seed orchards, but the main result of the model is that, within a certain range of heat-sum, the diurnal variation of airborne pollen can be explained on the basis of air humidity and wind speed. A good example showing the prediction power of the parameters included in the model is the night of May 31/June 1 (Fig. 3). During that night there was a peak in the curve of the measured pollen catch, as well as in the predicted curve, as a result of the lower air humidity than during other nights together with sufficient wind (about 2 $m \cdot s^{-1}$). The regular diurnal variation, i.e. a high amount of pollen during day time and a low amount at night, and its close relationship with air humidity, has been observed by SARVAS (1955). He presented an example similar to the present study; in a natural Scots pine stand, when the humidity was low the amount of pollen was high even at night (SARVAS 1962).

When the spatial variation in pollen density was investigated in details (Fig. 4), there was only slight variation during the first two days of anthesis (May 29 and 30), but the variation on the third and fourth day of anthesis (May 31 and June 1) was much larger. The variation in pollen density inside the seed orchard implies that the effect of wind and phenological differences in pollen shedding between the southern slope and the northern part of the orchard were of great importance (NIKKANEN 2001). On May 31, the highest pollen densities occurred on the southern slope, while on June 1, especially during the afternoon, they occurred in the northern part of the orchard. On this day, the highest densities were observed in the western, downwind side of the orchard, indicating heavy pollen shedding from the grafts as well as the effect of wind. The wind direction varied from day to day but, during the last two days of anthesis, it was mainly from the east (Table 1). The applied kriging method tends to give estimates close to the overall mean value when the distance to the nearest observation is large. The lack of samplers in the middle of the orchard has probably resulted in underestimates in the density maps for these areas (Figs. 2 and 4).

The average pollen density inside the seed orchard was about the same as outside during the first two days of anthesis, but on the third day it was much higher inside the orchard (Fig. 5). This seems to indicate that pollen caught inside the orchard during the first two days was mainly derived from outside the orchard, but on the third day, because of the higher pollen densities, it was from inside the orchard. This is in accordance with the results that the main proportion of pollen shedding from the seed orchard grafts took place within two days – from May 31 to June 2 (NIKKANEN 2001). Inside the seed orchard the pollen density increased considerably from the second to the third day, but outside it the greatest increase occurred one day later. This may indicate that there are some phenological differences in pollen shedding between the seed orchard grafts and the surrounding forest, as had been expected when seed orchards like the studied one, i.e. those of northern origin established at more southerly sites, were planned (SARVAS 1970).

The receptive period of female flowers and pollen shedding from male flowers occurred almost simultaneously in the orchard in 1995, while in 1992 and 1993 pollen shedding was more delayed (NIKKANEN 2001). However, the reproductive synchronisation was not complete even in 1995. The first female flowers became receptive on May 29, and all the flowers were receptive on May 31 (NIKKANEN 2001). Thus, on May 31 when abundant pollen shedding in the orchard started (Figs. 4 and 5), most of the female flowers had been receptive for one to two days, while some of them had already passed their receptivity (NIKKANEN 2001). This means that part of the orchard pollen was too late to pollinate the female flowers. This also confirms the presence of metandry (i.e. female flowers are receptive before male flowers shed pollen), which is characteristic for Norway spruce and Scots pine (SARVAS 1962, 1968), and is most pronounced in south-transferred seed orchards of these species (PULKKINEN 1994; NIKKANEN 2001).

The amount of airborne pollen was determined by means of two different types of pollen sampler. One of the samplers, the Sarvas-Wilska model, is a recording sampler with a clock mechanism that enables pollen to be trapped on one tape during a 1-week period. This makes it especially suitable for measuring temporal variation in airborne pollen. The rotorod sampler, on the other hand, was developed to measure the number of pollen grains or other small particles in a known volume of air, and is therefore suitable for measuring the density variation of pollen in the orchard. Because the rotorod sampler operates at a high rotation speed, it is only suitable for catching relatively small particles. However, according to our results, the size of a Norway spruce pollen grain is not too large to be caught using a rotorod sampler. In this study the Sarvas-Wilska sampler was found to give pollen quantities that agreed

almost exactly with the quantities caught with the rotorod sampler.

Flowering and cone production in the Heinämäki seed orchard was rather abundant in 1995, but the quality of the crop was poor; more than 90 % of the cones had resin flow and other forms of damage, while in 1989 damage was found in only 14 % of the cones (NIKKANEN 1992). The number of seeds extracted per cone was 22 in 1995, while in 1989 it was 87 (NIKKANEN, unpublished data). In addition to large differences in the quantity and quality of the seed among clones, there were also large and significant differences between different parts of the orchard (Table 2). For instance, cone production and 1000-seed weight were higher in the central and uppermost parts than in the other parts of the orchard, probably because of the more fertile soil (abandoned agricultural land) in this part (NIKKANEN & RUOTSALAINEN 2000). Clear spatial variation was also found in the number of seeds per cone and in the proportion of full seed. Because the proportion of full seed was strongly correlated with accumulated amount of pollen (Table 3), differences in the abundance of pollen may have affected this parameter.

The estimated rate of self-fertilisation in the seed orchard was 0.06 in 1995, which was higher than the rates estimated for the same seed orchard in 1989, 1992 and 1993, of 0.00, 0.04 and 0.00, respectively (PAKKANEN *et al.* 2000). However, the selfing estimate in 1995 was lower than that estimated by XIE and KNOWLES (1994) for a Norway spruce seed orchard in Canada (0.09), but higher than the estimate of PAULE *et al.* (1993) for two seed orchards in Sweden (outcrossing rates 0.95 and 0.98). The differences in the rate of selfing between the different parts of the orchard were not significant (Table 4).

The rate of pollen contamination in 1995 was 0.71, which is in the same level as the rates in the same seed orchard in 1989, 1992 and 1993 were 0.69, 0.69 and 0.71, respectively (PAKKANEN *et al.* 2000). In spite of the annual differences in the weather conditions and timing of flowering (NIKKANEN 2001), the contamination rates were surprisingly similar in different years. The rates were also high when they are compared to other studies concerning pollen contamination in Norway spruce seed orchards: PAULE *et al.* (1993) estimated contamination rates of 0.43 and 0.59 for two different seed orchards in Sweden. These orchards, which were also established with northern material, were located closer to the origin of the mother clones, and this may have had different effects on reproductive synchronisation. The contamination rates in Scots pine have varied from 0.45 to 0.76 (PAKKANEN & PULKKINEN 1991; WANG *et al.* 1991; YAZDANI & LINDGREN 1991). The method we used to estimate the pollen contamination is rather sensitive to differences in background pollen frequencies, because the detection probability of alien pollen is low in orchards that include a large number of clones. Even though the differences in isozyme gene frequencies are known to be small in Norway spruce populations (LUNDKVIST 1979), significant heterogeneity between pollen clouds has been reported (MUONA *et al.* 1990), and it is obvious that there is temporal variation in the gene frequencies of the pollen cloud during flowering and between years. However, because it is very difficult to obtain precise estimates of the gene frequencies of background pollen, some uncertainty has to be accepted (PAULE *et al.* 1993).

In pollen contamination significant differences were found between the different parts of the orchard (Table 4). In the middle of the orchard the contamination rate was about 0.50, while on the edges, except in the NW section, it was close to 0.90. The contamination was higher in the eastern than in the western parts of the orchard, obviously because of the prevailing wind direction during late anthesis. In a Scots pine seed orchard in Sweden, YAZDANI and LINDGREN (1991) reported significant differences between the blocks in the orchard, and also significant interaction between blocks and years.

The spatial variation in pollen contamination correlated significantly with the accumulated amount of airborne pollen (Table 3). Most of the differences in the pollen count between the sections occurred on the fourth day of anthesis (Fig. 4). In the middle and downwind side of the orchard the pollen densities were higher and the pollen contamination lower than in the other parts of the orchard. When differences in pollen density during late anthesis, or accumulated amount of pollen and wind direction, are taken into consideration, it is rather easy to understand the spatial variation in pollen contamination found in the study (Table 4).

The high rate of pollen contamination estimated in this study, and in the study of PAKKANEN *et al.* (2000), indicate that a strong gene flow into the seed orchards from outside sources is the predominant pattern at least in the orchards of northern origin established at more southern sites. This is also the case for Scots pine (PAKKANEN & PULKKINEN 1991; WANG *et al.* 1991; YAZDANI & LINDGREN 1991). Our results are in accordance with the findings of SORENSEN and WEBBER (1997), who also found that there are small but obviously effective quantities of pollen in the air before the shedding from orchard clones starts. The fact that the greatest increase in pollen density occurred outside the seed orchard one day later than inside (Fig. 5), indicating phenological differences between the orchard and

local forests, is one sign that the origin of airborne pollen during the first one or two days of flowering was probably not from the surrounding forests but mostly from more distant sources. The prevailing southerly winds on these days lend support to the possibility that pollen may have migrated from areas where the flowering of the species was in advance of that in the seed orchard area. The importance of long-distance pollen flight has been stressed by WHEELER *et al.* (1993), who reported that although the likelihood of gene flow is greatest when individuals are located close to each other and in phenological synchrony, long-distance pollen flight accounts for a considerable proportion of successful fertilisations in most seed orchards and natural stands. KOSKI (1970) pointed out the same, adding that even if the proportion of long-distance pollen remains small on average, it can account for a significant proportion of total pollination in some years.

The main results from this study were as follows: during the first two days of the receptive period in the seed orchard, most of the pollen caught was derived from outside the orchard, either from the surrounding forests or from more distant sources. Later the proportion of pollen shed from the seed orchard increased to a level above that of the background pollen. The phenological differences in pollen shedding between different parts of the orchard, and the wind direction, had an effect on spatial variation in pollen density. This variation, measured as the accumulated amount of pollen, had an effect on the rate of pollen contamination and probably also on the proportion of full seed.

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