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Functioning of a Norway spruce (*Picea abies* (L.) Karst.) seed orchard

Teijo Nikkanen

PUNKAHARJUN TUTKIMUSASEMA - PUNKAHARJU RESEARCH STATION

METLA

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Kuusen siemenviljelyksen toimivuus

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Esitetään Helsingin yliopiston maatalous-metsätieteellisen tiedekunnan luvalla julkisesti tarkastettavaksi Suomen metsämuseo ja metsätietokeskus Luston auditoriossa Punkaharjulla, keskiviikkona 12. päivänä kesäkuuta 2002 kello 12.

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Abstract

The overall objective of the thesis was to study the functioning of a Norway spruce seed orchard and, in more detail, to investigate the clonal balance and synchrony of reproduction, and aspects of mating patterns and their effects on genetic diversity and quality of the seed crop.

All the data for the thesis have been collected from Norway spruce seed orchard no. 170 (Heinämäki), established in 1968 at Korpilahti (62°13'N, 25°24'E, 160–190 m asl). The orchard consists of 67 clones originating from latitudes 64°–67°N. The grafts in the orchard (13.2 ha in area) have been planted using a clonal-row design.

The between-year variation in both female and male flowering was large, and during the 13-year study period there were 6 abundant flowering years. Differences among the clones were large, and correlations between the clones in different years were usually positive and significant. The average date of flowering varied by three weeks, and it was better predicted by effective temperature sum than by the calendar day. The receptive period started normally about one day earlier than anthesis, and it lasted from 5 to 8 days. In general, the flowering periods of the clones overlapped. The differences in receptivity were genetically determined, while pollen shedding was more affected by environmental factors.

Temporal and spatial variation in airborne pollen were large. Pollen densities inside and outside the orchard were about the same in the beginning of flowering, but later on the density was higher in the orchard, and showed strong spatial variation. The rate of pollen contamination was about 0.70 in all the four years studied. The contamination between the different parts of the orchard varied from 0.51 to 0.87, the lowest rate being estimated for the middle section and the highest rate for the edges of the orchard. The rate of self-fertilisation varied annually from 0.00 to 0.06, with no spatial variation. Thus, cross-fertilisation within the orchard clones remained very low, varying from 0.23 to 0.31. The paternal success among the clones, studied using controlled crossings, was unequal.

The effective number of clones in the seed orchard of 67 clones was 56 when the variation in ramet number was taken into account. After adjusting for the variation in both female and male flowering, in addition to ramet variation, the effective clone number for the imaginary seed crop was 32 on the average, with a large annual variation. The pollen contamination of 0.70 increased the number, and thus the genetic diversity, by two-fold. Cone and seed damage lowered the effective clone number.

The most important result of the thesis was that pollen contamination in Norway spruce seed orchards is very difficult to avoid, and thus its influence on adaptability, genetic diversity and genetic gain has to be taken into consideration when seed orchards are planned or utilised.

Keywords: flowering, pollination, reproductive phenology, pollen competition, mating patterns, genetic diversity

Seloste

Kuusen siemenviljelyksen toimivuus

Tämän väitöskirjatutkimuksen tavoitteena on ollut selvittää, kuinka hyvin siemenviljelyksille asetetut teoreettiset tavoitteet tasasuhtaisesta kukinnasta ja sisäisestä pölytyksestä täyttyvät siementuotantovaiheen saavuttaneessa kuusen siemenviljelyksessä. Tätä varten kuusen siemenviljelyksen toimivuutta on tutkittu monipuolisesti. Tarkasteltavana on ollut kukintarunsauden vuotuinen ja kloonien välinen vaihtelu sekä kukinnan ajoittuminen, siitepölyn määrä viljelyksellä ja sen ulkopuolella sekä syntyneen siemenen geneettinen kokoonpano. Lisäksi on tutkittu siitepölykilpailua.

Kaikki väitöskirjatutkimusta varten kerätty aineisto on peräisin kuusen siemenviljelykseltä nro 170 (Heinämäki), joka on perustettu vuonna 1968 Korpilahdelle (62°13'P, 25°24'I). Siemenviljelys koostuu 67 kloonista, jotka ovat peräisin Pohjois-Suomesta (64°–67°). Viljelys on kooltaan 13,2 ha ja se sijaitsee puoliksi vanhalla mäkipellolla (160–190 m mpy), joka viettää loivasti etelään ja jyrkästi itään ja länteen. Viljelys on perustettu istuttamalla vartteet 3,5 x 6,5 m:n välein niin että saman kloonin vartteet ovat riveissä 6,5 m:n päässä toisistaan. Viljelystä on harvennettu kahdessa vaiheessa; puolet vuonna 1987 ja loput vuonna 1994.

Emi- että hedekukinnan määrä arvioitiin viljelyksellä vuosina 1984-1996. Kukinnan ajoittumista mitattiin siitepölymittareiden avulla vuosina 1984-1995 ja sitä havainnoitiin vartteista silmävaraisesti vuosina 1989, 1992, 1993 ja 1995. Lisäksi vuonna 1995 mitattiin ilmassa olevan siitepölyn määrän alueellista vaihtelua viljelyksellä ja sen lähiympäristössä. Viljelykseltä kerättiin siementä taustapölytysanalyysejä varten vuosina 1989, 1992, 1993 ja 1995 sekä kloonikohtaisen siementuotannon määrän ja laadun selvittämistä varten vuosina 1989 ja 1995. Viljelyksen kaikista klooneista kerättiin vuosina 1996 ja 1998 siitepölynäytteet siitepölyn itämistehokkuuden selvittämistä varten. Lisäksi vuonna 1998 tutkittiin siitepölykilpailua valvottujen paristeytysten avulla. Siemenviljelys myös kartoitettiin, tehtiin maaperästä viljavuusanalyysit, havainnoitiin sääoloja fenologia- ja pölytystutkimusten yhteydessä sekä mitattiin vuosittain kukinnan laskennassa olleiden vartteiden koko.

Vuosien välinen vaihtelu kukinnan runsaudessa oli suuri: 13-vuotisen tutkimusjakson aikana kukinta oli 6 vuotena melko runsas, 5 vuotena heikko ja 2 vuotena ei emikukintaa ollut ollenkaan. Kloonien väliset erot kukinnan runsaudessa olivat suuret ja vuosien välinen korrelaatio kloonien kesken oli yleensä positiivinen ja tilastollisesti merkitsevä. Kukinnan ajankohta vaihteli kolme viikkoa, mutta se pystyttiin ennustamaan melko tarkasti lämpösumman perusteella. Emikukat aukenivat keskimäärin päivää aikaisemmin kuin saman vartteen hedekukista siitepöly alkoi varista. Emikukat olivat viljelyksellä auki 5-8 päivää. Suurin osa klooneista kukki ainakin osittain yhtäaikaisesti. Emikukinnan ajoittuminen oli voimakkaammin geneettisesti

säädelty kuin hedekukinnan; siitepölyn varisemiseen vaikuttivat ympäristötekijät enemmän.

Ilmassa olevan siitepölyn määrä vaihteli sekä ajallisesti että paikallisesti. Kukinnan alkuvaiheessa siemenviljelyksellä ei ollut sen enempää siitepölyä kuin sen ulkopuolellakaan, mutta pari päivää myöhemmin, sen jälkeen kun siitepölyn irtoaminen vartteista oli kunnolla alkanut, nousi siitepölypitoisuus viljelyksellä selvästi ympäröiviä alueita suuremmaksi. Samalla siitepölyn määrä viljelyksen eri osissa vaihteli voimakkaasti; ensin pölyä oli eniten viljelyksen etelärinteellä ja myöhemmin viljelyksen pohjoisosissa tuulen suunnan vaikuttaessa eroihin. Taustapölyttyneen siemenen osuus oli noin 70 % kaikkina niinä 4 vuotena kun sitä tutkittiin. Siinä oli kuitenkin suuria alueellisia eroja; vähiten (51 %) taustapölyttynyttä siementä oli viljelyksen keskellä ja eniten (87 %) sen reunoilla. Itsepölytyssiemenen osuus vaihteli viljelyksellä vuosittain 0:sta 6 %:iin. Viljelyksellä syntyneen ristipölytyssiemenen osuus jäi hyvin alhaiseksi vaihdellen 23:sta 31 %:iin. Lisäksi todettiin, että siitepölykilpailu voi vaikuttaa viljelyksellä syntyneen siemenen geneettiseen kokoonpanoon.

Siemenviljelyksen ja sen siemensadon geneettistä monimuotoisuutta arvioitiin tehoisan klooniluvun avulla. Tehoisa klooniluku vastaa niiden, toisilleen ei sukua olevien kloonien lukumäärää, jotka osallistuvat yhtä suurilla osuuksilla siemenen tuottamiseen. Viljelyksen kloonimäärän ollessa 67 tehoisa klooniluku oli 56, kun kloonien välinen vaihtelu vartemäärässä otettiin huomioon. Kun tarkasteluun lisättiin emi- ja hedekukinnan vaihtelu, aleni siemensadolle laskettu klooniluku keskimäärin 32:een, vuosien välisen vaihtelun ollessa suuri. Taustapölytys lisää geneettistä monimuotoisuutta; viljelykseltä mitattu 70 %:n taustapölytys kaksinkertaisti tehoisan klooniluvun. Käpy- ja siementuhot taas näyttivät alentavan siemensadon monimuotoisuutta.

Tutkimuksen kohteena ollut kuusen siemenviljelys ei täytä kaikkia niitä teoreettisia tavoitteita, joita siemenviljelyksille on asetettu. Kaikki kloonit eivät kuki yhtä runsaasti; kukinnan perusteella laskettu tehoisa klooniluku jää puoleen kloonimäärästä. Myös kloonien välinen pölytyskilpailu ja erot tuhoalttiudessa sekä jossain määrin kukinnan eriaikaisuus vaikuttavat siihen, että kloonit eivät osallistu samalla painolla siemensadon muodostamiseen. Odotettua runsaampi taustapölytys poikkesi kuitenkin kaikkein eniten niistä tavoitteista, jotka viljelykselle oli asetettu. Sen vaikutus siemenviljelyksen tuottaman siemenen sopeutumiskykyyn, jalostushyötyyn ja geneettiseen monimuotoisuuteen on otettava huomioon kun nykyisiä viljelyksiä hyödynnetään ja uusia suunnitellaan.

Avainsanat: kukinta, pölytys, kukinnan fenologia, siitepölykilpailu, pariutumistavat, geneettinen monimuotoisuus

Alkusanat

Aloitin siemenviljelysten tutkimisen 1980-luvun alussa, kun prof. Max. Hagman ja Veikko Koski siirsivät minulle vaativan perinnön metsäpuiden lisääntymis- ja siemenviljelystutkimusten jatkamisesta. Samalla tuli esille, että erityisesti kuusi kaipaa tutkimusta; mäntyviljelyksistä tiedettiin jo jotakin. Kuusiviljelysten tarkastuksessa selvisi pian, että Heinämäen siemenviljelys Korpilahdella olisi sopiva tutkimuskohde. Se kukki jo tuolloin hyvin ja lisäksi siellä voitaisiin tutkia kuusen siemenviljelysten toimivuutta monipuolisesti; Heinämäellä olivat kaikki ongelmat samalla paikalla. Viljelys sijaitsi keskellä laajoja kuusimetsiä, se oli perustettu istuttamalla vartteet klooniriveihin ja se oli pohjoista alkuperää, mutta sijaitsi etelässä.

Tämä väitöskirja on tehty Metsäntutkimuslaitoksen Punkaharjun tutkimusasemalla. Kiitän asemanjohtaja Juhani Häggmania ja aseman henkilökuntaa kaikesta saamastani avusta ja tuesta. Työtä ei kuitenkaan tehty yksin punkaharjulaisten voimin; Metsähallituksen Jyväskylän yksikön apu on ollut ratkaiseva. Ilman Metsähallituksen työvoima-apua, jota 1980-luvulla Arvo Leppänen ja myöhemmin Kari Lahtinen ja Tapani Relander järjestivät, olisivat monet tärkeät mittaukset jääneet tekemättä. Lisäksi viljelystä harvennettiin ja lähimetsien puut kaadettiin, aina kun tutkimus sitä vaati. Haluan kiittää kaikkia, jotka ovat näihin töihin osallistuneet.

Kenttätöitä Heinämäellä on kuitenkin koko ajan johdettu Punkaharjulta käsin. Alusta saakka tätä vastuuta on kantanut Esko Oksa, välillä Pentti Manninen. Lisäksi mukana ovat olleet Eija Matikainen, Tarja Salminen, Jussi Tiainen, Auvo Harinen, Heikki Paajanen, Jouko Lehto ja Matti Tolvanen. Heille kaikille esitän suuret kiitokset, samoin kuin Tiina Tynkkyselle, jonka apu aineistojen käsittelyssä ja julkaisujen laadinnassa on ollut korvaamaton.

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Lopuksi haluan kiittää vaimoani Kaijaa kärsivällisyydestä ja myötäelämisestä ja lapsiani Samulia, Jenniä ja Sannia kannustuksesta ja joustamisesta. Se kaikki on ollut tarpeen.

Punkaharjulla toukokuussa 2002 Teijo Nikkanen

Preface

I started to work with seed orchards in the beginning of the 1980's, when Prof. Max. Hagman and Veikko Koski transferred reproduction and seed-orchard research to my responsibility. At the same time it became apparent that spruce especially needed to be studied in more detail. After an inventory of the Finnish spruce seed orchards, it was decided that the Heinämäki orchard at Korpilahti, Central Finland, would be an excellent subject for the research; at that time it was already flowering well, but had all the problems connected with spruce seed orchards.

This thesis has been carried out at the Punkaharju Research Station. I am grateful to the head, Dr. Juhani Häggman, and to the staff of the Station for all the help and stimulation I have received. The work has not been performed by the staff of Punkaharju alone, and the help of the Forest and Park Service at Jyväskylä has been essential. Many of the measurements could not have been made without the assistance of the Service's staff, which was provided in the 1980's by Arvo Leppänen, and later on by Kari Lahtinen and Tapani Relander. I am very grateful to them, and all the other persons who have participated in the work during the course of the research.

All the fieldwork at Heinämäki was, however, organised from Punkaharju, with the assistance of the staff of the Punkaharju Research Station. I am extremely grateful to Esko Oksa and Pentti Manninen for organising the work, and Eija Matikainen, Tarja Salminen, Jussi Tiainen, Auvo Harinen, Heikki Paajanen, Jouko Lehto and Matti Tolvanen for participating in the various work stages at both Heinämäki and Punkaharju. I would also like to thank Tiina Tynkkynen, whose assistance in analysing the data and preparing the manuscript has been very important.

I am grateful to all my co-authors in the individual studies, Seppo Ruotsalainen, Tuija Aronen, Hely Häggman, Martti Venäläinen, Anni Harju, Heidi Tiimonen, Anne Pakkanen, Pertti Pulkkinen and Jaakko Heinonen for their collaboration. Especially I would like to thank Tuija Aronen and Seppo Ruotsalainen for their valuable comments on the manuscript of the thesis. In addition, I would like to thank John Derome for checking the language of both the thesis and its individual studies. I would also like to thank Dr. Bo Karlsson, Doc. Markku Nygren and Doc. Pertti Pulkkinen for their valuable advice on the thesis. In addition, I am very grateful to Prof. Peter Tigerstedt from the University of Helsinki, for his continuous support during my studies.

Finally, I would like to thank my wife Kaija, for being so patient and understanding, and my children Samuli, Jenni and Sanni, for believing all the time that it would be soon finished.

Punkaharju, May 2002 Teijo Nikkanen

List of original publications

This thesis is based of the following publications, which are referred to in the text by their Roman numerals. All the publications are reprinted with the permission of the publishers.

I Nikkanen, T. & Ruotsalainen, S. 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34(3): 205-222.

II Nikkanen, T. 2001. Reproductive phenology in a Norway spruce seed orchard. Silva Fennica 35(1): 39-53.

III Nikkanen, T., Aronen, T., Häggman, H. & Venäläinen, M. 2000. Variation in pollen viability among *Picea abies* genotypes – potential for unequal paternal success. Theoretical and Applied Genetics 101(4): 511-518.

IV Aronen, T., Nikkanen, T., Harju, A., Tiimonen, H. & Häggman, H. 2002. Pollen competition and seed-siring success in *Picea abies*. Theoretical and Applied Genetics 104(4): 638-642.

V Pakkanen, A., Nikkanen, T. & Pulkkinen, P. 2000. Annual variation in pollen contamination and outcrossing in a *Picea abies* seed orchard. Scandinavian Journal of Forest Research 15(4): 399-404.

VI Nikkanen, T., Pakkanen, A. & Heinonen, J. 2002. Temporal and spatial variation in airborne pollen and quality of the seed crop in a Norway spruce seed orchard. Submitted.

Nikkanen planned the work in studies **I**, **II**, **IV** and **VI** alone, and together with co-authors in **III** and **V**. Nikkanen was responsible for collecting the field data in all studies, and he made the data analyses alone or mainly in **I** (genetic diversity with Ruotsalainen), **II**, **III** and **VI** (variation in airborne pollen with Heinonen). Nikkanen wrote the manuscript alone or mainly in **I**, **II** and **VI**, jointly with Aronen in **III**, participated in writing in **IV** and **V**. Isozyme analyses were performed by Harju and Tiimonen in **IV**, and by Pakkanen in **V** and **VI**.

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Introduction

1.1 Norway spruce as a forest tree

Norway spruce, *Picea abies* (L.) Karst., belongs to the genus *Picea*, which includes 34 species, all distributed in the northern hemisphere (Farjon 1990). *Picea* is one of the largest and most widely spread genus in the family of *Pinaceae,* and the genus is the most important constituent of the northern coniferous forests (Sarvas 1964, Farjon 1990).

Picea abies is supposed to have originated in East Asia in prehistorical times, and to have subsequently migrated to Europe through Siberia (Schmidt-Vogt 1977). During the last Ice Age the species is assumed to have survived in Europe in three refugia: the Dinaric Alps, the Carpathians and North-Central Russia (Schmidt-Vogt 1977, Lagercrantz and Ryman 1990). *Picea abies* migrated from these refugia into its present natural distribution areas; the northeast European origins migrated from the Russian refugium. Migration into Finland and Fennoscandia took place through Carelia, and it passed through Finland into Scandinavia during the period 5500–2500 BP (Moe 1970, Tolonen 1983).

The natural distribution of *Picea abies*, ranging from the Atlantic coast in Norway (12°E) across Fennoscandia, North Russia and Siberia to the Sea of Ohotsk (154°E), comprises an 8000 km long, virtually unbroken area (Sarvas 1964). The distribution area stretches from the mouth of Khatanga River (72°N) in the north to the Balkan Peninsula (41°N) in the south. The Central and Southeast European distribution areas lie outside the continuous North Eurasian area (Sarvas 1964, Schmidt-Vogt 1977), and they are clearly found to differ both in quantitative and molecular traits (Collignon et al. 2002). In addition, *Picea abies* has been cultivated in Central Europe more than 300 years, and it has also been planted outside its natural range (Schmidt-Vogt 1977).

In Europe, *Picea abies* is economically the most important coniferous tree species (Consensus document ... 1999). It provides raw material for both the chemical and mechanical forest industries. In Finland, *Picea abies* has been considered as the second-most important species after Scots pine, *Pinus sylvestris* (L.), but in many respects the importance of spruce has recently increased; the growing stock drain (29 mill. m^3 in 2000), as well as the number of seedlings planted annually (81.5 mill. in 2000), are larger than those for pine. Seeding and planting were used on about 70% (117 000 ha) of the total forest regeneration area in Finland. The proportion of pine out of this area was 49% (46% planted), and of spruce 41% (97% planted) (Metsätilastollinen vuosikirja 2001).

1.2 Genetic variability

Genetic variation is the basis of the maintenance and long-term stability of forest ecosystems since the amount and pattern of genetic variation determine the ability of forest tree species to adapt to variable environmental conditions (Hamrick et al. 1992, Müller-Starck et al. 1992). The wide genetic variation found in natural forests is also the basis for forest tree breeding, and adequate genetic diversity in forest regeneration material is the basic requirement for guaranteeing the adaptability of cultivated future forests. Genetic variation here means genetically determined phenotypic variation, and genetic diversity the genetic variability at the genotypic level (Ruotsalainen 2002).

Genetic variation is affected by several, partly counteracting, evolutionary factors (see Eriksson and Ekberg 2001). Natural selection, genetic drift and mutations increase differentiation among populations, while gene flow reduces it. The effect of these factors within a population is differing; gene flow is now the main factor increasing the variation, while inbreeding and genetic drift reduce it. In addition, mutations also increase the variation and natural selection reduces it. In *Picea abies*, as well as in other species with a large population size and clinal variation, genetic drift is negligible. Gene flow, on the other hand, is an important factor in reducing differentiation among populations while, at the same time, bringing new gene material into a population. Mutations also tend to increase genetic variability but, because mutation rates in trees are very low, their immediate contribution to the variation is small.

The role of phenotypic plasticity in genetic differentiation is ambiguous. It is generally accepted that phenotypic plasticity may contribute to the fitness of a genotype, and it could therefore be favoured by natural selection. Phenotypic plasticity in *Picea abies* is expected to be large (Eriksson and Ekberg 2001).

The genetic variation of forest trees can be considered at different levels; variation within species, among populations, and among individuals within a population. Because the concept of a population is often difficult to define, particularly in wind-pollinated trees, the units of provenance, stand, and tree are sometimes used instead. At the species level genetic variation in most of the adaptive traits of forest trees is clinal, i.e. the population means change gradually with geographic distance. This variation is caused by effective gene flow and gradually changing selection pressure (Eriksson and Ekberg 2001). The specific evolutionary history of each species plays an important role in determining the patterns of genetic variation (Lagercrantz and Ryman 1990, Hamrick et al. 1992, Collignon et al. 2002).

Trees maintain more variation within species and within populations than other plants, but have less variation among populations (Hamrick et al. 1992). It is also known that the largest proportion of genetic variation in most forest trees within a geographically limited area is among trees within stands (Eriksson et al. 1987, Simpson 1998, Ruotsalainen et al. 2002).

Most boreal-temperate conifer populations are highly variable at isozyme loci, but there is little differentiation among populations due to efficient gene flow caused by pollen dispersal (Tigerstedt 1973, Muona 1990). Instead, populations are much more differentiated in adaptive quantitative traits, showing clinal geographic variation, due to selection pressure, while isozyme variability indicates that isozyme loci are not subject to the same selection pressures (Muona 1990). *Picea abies* and *Pinus sylvestris* are good examples of species that are highly differentiated between latitudes and altitudes with respect to quantitative traits related to climatic constraints. In *Picea abies* the clinal variation in growth rhythm, as well as in growth and survival, has been extensively investigated (Heikinheimo 1949, Hagman 1980, Koski 1989, Beuker 1994a, 1994b, Danusevicius and Persson 1998, Hannerz 1999), and in forest cultivation this is a widely utilised character of the species (Pitkäntähtäyksen metsänjalostusohjelma... 1988, Karlsson and Rosvall 1993, Rosvall et al. 1998).

Picea abies is morphologically one of the most variable tree species (Schmidt-Vogt 1977). Its large genetic variation in various quantitative and qualitative traits can be divided into variation among provenances, and variation among individuals within a provenance or population. In spite of the wide-ranging natural distribution of the species, Lagercrantz and Ryman (1990) found in an isozyme study that only 5% of the total genetic diversity was explained by differences among provenances. Differentiation among populations was even smaller than that among provenances, the most part of the variation being among individuals. The great genetic variation in *Picea abies* forests is affected by a large number of factors: the ancient origin and immigration history of the species, natural selection, and an extensive gene flow caused by effective pollen distribution and, probably to a lesser extent, by human activities and genetic drift in small populations (Lagercrantz and Ryman 1990).

For estimating genetic diversity in natural populations (Wright 1931) introduced the concept of effective population size. Since then the concept has been developed and applied by many population geneticists and plant breeders, with the main focus on two alternative aspects: the inbreeding effective population number and the variance effective population number (Crow and Kimura 1970, Crow and Denniston 1988, Muona and Harju 1989, Caballero 1994, Burczyk 1996, Kjær 1996). Because effective population size describes the rate of change in a

population, Lindgren et al. (1996, 1997) developed the concept of status number (effective clone number), which is a more functional measure for the state of a non-changing population, e.g. a seed orchard crop. The application of status number for estimating the genetic diversity of seed orchards or seed orchard crops has been discussed by Kjær and Wellendorf (1996, 1997), Lindgren and Mullin (1998), Kang and Lindgren (1998), Ruotsalainen et al. (2000), and it will be continued in this thesis.

1.3 Reproduction

Picea abies is a typical coniferous tree species, monoecious and wind-pollinated. Its female flowers are generally located at the top of the shoot in the upper part of the crown, and male flowers at the base of the preceding year's shoot in the lower part of the crown. In this thesis and in the individual studies the reproductive organs are generally called flowers although their organological status is somewhat debatable (Sedgley and Griffin 1989).

Picea abies has a 2-year-long reproductive cycle, which is the shortest cycle found in boreal and temperate forest trees (Owens and Blake 1985). The other common type of cycle, found for instance in *Pinus sylvestris*, takes three years. In the 2-year cycle the initiation and development of male and female buds occur during the growing season prior to flowering and pollination. Pollination occurs in the spring or early summer of the second year, and fertilisation a few weeks after pollination. Following fertilisation, embryo and seed development are rapid and continuous. The seeds are mature and ready to be released in the late summer or autumn of the pollination year. The different stages of the reproductive cycle are affected and regulated by climatic factors (Owens and Blake 1985).

Reproductive buds are formed through the transition of an indeterminant vegetative apex into a determinant floral apex (Owens and Blake 1985). Floral initiation and development of reproductive buds are favoured by high temperatures during the growing season in many species in different climatic conditions, as is the case for *Picea abies* in our conditions (Lindgren et al. 1977, Pukkala 1987). The timing of bud determination has been found to be very similar for many *Picea* species (Owens and Blake 1985), and it occurs at a time close to the termination of lateral shoot elongation. Following differentiation, the development of reproductive buds is completed well before winter dormancy, the male flower buds usually terminating earlier than the female flower buds. In all boreal-temperate conifers, pollen sacs are initiated within the male flower buds before winter dormancy (Owens and Blake 1985).

Male meiosis and pollen development take place in spring, and are regulated by the day length and temperature sum (Luomajoki 1986). In *Picea abies*, as in other *Picea* species, meiosis starts soon after the end of winter dormancy (Sarvas 1972, 1974), which in the climate of southern Finland means early May (Luomajoki 1982). Meiosis and pollen development take only a few weeks, and end at anthesis. Female meiosis usually starts just before the female strobili become receptive (Sarvas 1968).

Pollination, excluding floral initiation, is probably the most decisive part of the reproductive cycle in many forest trees (Owens and Blake 1985). In wind-pollinated conifers it can be divided into pollen shedding, pollen distribution, and pollen capture. The timing of pollen shedding is regulated by weather conditions, the effective temperature sum being the main factor in *Picea abies* (Sarvas 1968). Pollen shedding in an individual tree usually takes place slightly later than when the female strobili become receptive (Sarvas 1962, 1968). This feature, called metandry, is a strategy of many wind-pollinated conifers to avoid self-pollination. However, the rate of self-pollination in natural stands of *Picea abies* and *Pinus sylvestris* usually ranges from 10 to 20%, and in extensive forested areas, one half of the pollen comes from trees growing less than 50 m away (Koski 1970). Gene flow through pollen distribution from neighbouring stands or even further way is of crucial importance, and in some years it can account for a significant proportion of the total pollination (Koski 1970, Wheeler et al. 1993). Although distribution of pollen is a widely studied issue (Lanner 1966, Koski 1970, Sorensen 1972, Di-Giovanni and Kevan 1991, Wheeler et al. 1993), more information is needed.

The mechanism of pollen capture and entrance of pollen into the ovules of conifers has been investigated extensively in many species (Doyle 1945, Sarvas 1962, Singh 1978, Owens 1980), including *Picea abies* (Sarvas 1968). In *Picea* a pollen grain is transferred from the mouth of the micropyle into the pollen chamber with the aid of a pollination drop (Sarvas 1968, Owens 1993, Owens et al. 1998). Sarvas (1968) found that the average capacity of a pollen chamber in *Picea abies* is 5 pollen grains but, even after abundant pollination, the chambers are not always full. Of the factors preventing complete pollination, Sarvas (1968) reported that wind that frequently blows from the same direction during flowering is the worst. The rain, if it does not continue for days, is not as harmful. Spring frosts, which disturb the pollination drop mechanism, can considerably decrease the success of pollination (Sarvas 1968).

Fertilisation in *Picea abies* occurs 3-4 weeks after pollination (Sarvas 1968, Christiansen 1972). During the stage from pollination to fertilisation, the pollen grains germinate and pollen tubes penetrate through the nucellus into the ovule. The germination capacity of *Picea abies* pollen in its natural environment, i.e. at the tip of the nucellus, varies from 86 to

99% (Sarvas 1968). It is essential for the reproductive strategy of the species that there is usually more than one archeogonium per ovule, three being the average in natural stands in southern Finland (Sarvas 1968). In addition, the fact that the number of pollen grains in a pollen chamber might be larger than the number of archeogonium per ovule provides an opportunity for male gametophyte competition already before fertilisation. During or soon after fertilisation, embryo abortion eliminates the genotypes that are homozygous for lethal or sublethal genes. Koski (1973) has estimated an individual *Picea abies* tree contains an average of 10 (from 2 to 20) embryonic lethals. Later on, the competition between developing zygotes reduces the number of embryos to one per ovule. According to Sarvas (1968), embryo abortion occurs in 20 to 40% of all fertilisations, and competition between the vigorous embryos continues in about 70% of the ovules. In *Picea abies* this pattern, called polyzygotic polyembryony, reduces the rate of inbreeding, and in many conifers it is equivalent to the self-incompatibility pattern common in angiosperms (Hagman 1975). Koski (1973) has estimated, using embryonic lethals, that nearly 90% of the inbred embryos are destroyed before the seed is mature, thus reducing the rate of self-fertilisation by 5 to 10%.

In southern Finland fertilised ovules of *Picea abies* develop into anatomically mature seeds within about two months (Mikkola 1969). Seed maturation is a problem only in northern Finland, where the temperature sum required for the complete maturation of seed is not reached every year (Kujala 1927, Henttonen et al. 1986). A much greater problem in southern Finland is cone and seed damage, caused by several rust and insect pest species (Rummukainen 1960).

Seeds of *Picea abies* are usually shed early in the spring and, because of the snow cover that is often still present at that time of the year, the seeds may be dispersed by wind over long distances (Heikinheimo 1932). However, most of the seeds remain close to the mother tree, and gene flow through seed dispersal cannot be regarded as very efficient. Although *Picea abies* is a shade-tolerant and competitive species and thus occupies new areas rather easily, the most productive spruce forests with dense ground vegetation pose problems for natural regeneration.

In natural forests of *Picea abies* the between-year variation in the abundance of flowering and seed crop is large (Blomqvist 1876, Heikinheimo 1932, 1948, Tirén 1935, Koski and Tallqvist 1978). The periodicity of abundant flowering is irregular, and the occurrence of good flowering years is the more seldom, the more northern is the region in question (Koski and Tallqvist 1978). The variation in flowering is also large between trees within a stand (Sarvas 1968, Koski and Tallqvist 1978).

1.4 Production of genetically improved seed

The genetic improvement of forest trees is based on exploitation of the genetic variation, occurring in natural forests, in economically important traits (Wright 1976). Phenotypic selection of superior trees, so called plus trees, has generally been the first phase of forest tree breeding, followed at a later stage by the progeny testing of plus trees and establishment of clonal seed orchards. In many countries the first seed orchards have been established soon after the start of plus tree selection, while large-scale progeny testing has started at a later stage, often on the basis of the seed collected from the seed orchards (Zobel and Talbert 1984). Establishing the first seed orchards soon after enough plus tree material has become available has made it possible to produce improved seed, although not yet genetically tested, within a reasonable time.

In Finland forest tree breeding focused during the first two decades (it started in 1947) almost solely on the phenotypic selection of plus trees (Oskarsson 1995). A total of 770 Norway spruce plus trees were selected during 1947-1971; the number of Scots pine plus trees selected during the same period was, however, 6526 (Nikkanen et al. 1999). Although the first seed orchards were established in the early 1950's, and have continued on a small scale ever since, the major activities in this field did not start until more than a decade later. In 1963 an official report (Lausunto maamme... 1963) was issued about the total area of seed orchards required for the production of genetically improved reforestation seed in Finland. The large-scale realization of seed orchards started some years later, and was completed by the mid 1970's (Nikkanen et al. 1999). Large-scale progeny testing started in the late 1960's, and continued through the 1970's and 1980's up until the mid 1990's (Häggman and Oksa 1999, Yrjänä et al. 2000). The number of Scots pine progeny tests planted so far is much greater (1394 trials totalling 2100 ha) than that of Norway spruce (246 trials, 280 ha). The spruce progeny tests are also much younger as they were mainly planted in the 1980's and early 1990's.

Since 1967 the forest tree breeding activities have been based on 10-year programs (Mikola 1992). In addition, a new seed orchard program for 1990-2025 (Metsäpuiden siemenviljelysohjelma... 1989) was carried out simultaneously with the revision of the most recent tree-breeding program (Pitkäntähtäyksen metsänjalostusohjelma... 1988). The seed orchard program was revised in 1997 (Männyn, kuusen ja ... 1997).

The aim of an ideal seed orchard is to produce genetically superior, frequent, abundant and easily harvested seed crops, and an orchard has to fulfil certain requirements with respect to flowering and pollination (Zobel et al. 1958, Sarvas 1970, Faulkner 1975, Werner 1975). These

requirements, reproductive synchronisation, balanced flower production, random mating, minimal selfing and isolation from non-orchard pollen sources, are the basic factors affecting the functioning of wind-pollinated seed orchards (Sweet 1975, Koski 1980, Blush et al. 1993). The functioning of seed orchards is, however, often far from ideal. There are large clonal and annual differences in flowering abundance in several species (Sweet 1975, Jonsson et al. 1976, Bhumibhamon 1978), including *Picea abies* (Skrøppa and Tutturen 1985, Ruotsalainen and Nikkanen 1989, Kjaer and Wellendorf 1997). The variation in reproductive phenology also has an effect on the genetic composition of the seed produced in seed orchards (Chung 1981b, Blush et al. 1993, Harju and Nikkanen 1996). Owing to the abundance of the same species in adjacent forests and effective pollen distribution (Koski 1970, Lindgren et al. 1995), high pollen contamination has proved to be a serious problem in both Scots pine and Norway spruce seed orchards (El-Kassaby et al. 1989, Harju and Muona 1989, Pakkanen and Pulkkinen 1991, Savolainen 1991, Paule et al. 1993). In addition, the genetic composition of the seed-orchard crops may be affected by competition among pollen grains.

In Finland the seed orchards have been established using clones originating from geographically and climatically limited areas (Sarvas 1970, Koski 1980, Nikkanen et al. 1999). This was done in order to ensure the adaptability of the seed orchard material to its utilisation area, which was usually planned to be the same as that of the clone origins. The aim of limiting the origin was also to ensure simultaneous flowering of the seed orchard clones. Another measure directed at the reproductive synchronisation of the seed orchards was to locate the orchards of northern origin in the southern parts of the country. In addition to enhanced flowering and better seed maturation, this was done in order to achieve phenological isolation between the seed orchard clones and surrounding forests (Sarvas 1970). The hypothesis was that the temperature sum required for the onset of flowering in trees adapted to northern conditions would be smaller than that in trees adapted to more southern conditions (Sarvas 1962, 1968, 1970). However, no phenological isolation has been found in Scots pine (Pakkanen and Pulkkinen 1991, Pulkkinen 1994b), and no results from this have been reported for Norway spruce seed orchards.

The Norway spruce seed orchards, which are now in the seed-producing phase in Finland, were established during 1965–1972. The number of such orchards is 23, and their total area is 276 hectares (Nikkanen et al. 1999). All of them have been established by grafting, and they consist of 601 plus tree clones. The average number of clones per seed orchard is 76 (35 to 196 clones), and the average size of the orchards is 12 hectares (2.8 to 30 ha). The orchards have been established using two spacing alternatives: 5 x 5 m or 3.5 x 7 m (400 grafts/ha in both

cases). The spruce seed orchards in Finland have one feature lacking from the pine seed orchards; the grafts in two-thirds of the orchards have been planted using a clonal-row design instead of a randomised design.

The long juvenile phase, typical for a climax species like *Picea abies* (Chalupka and Cecich 1997, Almqvist 2001), has caused delays in the onset of seed production and effective utilisation of the spruce seed orchards. However, most of the seed used nowadays in Finland for the artificial regeneration of spruce is produced in seed orchards. The proportion of spruce seed-orchard seed used in nurseries increased rapidly during the 1990's; in 1991 it was only 10%, but in 2000 as much as 70% (Metsätilastollinen vuosikirja 2001). At the same time, the proportion of pine seed-orchard seed has remained at a level of about 60%. However, seed-orchard seed has not been used in the same quantities in all parts of the country; while almost all the nursery seed for southern Finland has, in recent years, been of seed orchard origin, very little seed-orchard seed has been available for northern Finland.

The reasons for the lack of pine seed-orchard seed in northern Finland have been the pollination of south-transferred seed orchards by non-orchard southern pollen (Pakkanen and Pulkkinen 1991), and the poor adaptability of this kind of provenance hybrid seed in the locations of the mother clones (Nikkanen 1982, Mikola 1993b, Pulkkinen et al. 1995).

The progeny test results of spruce in northern Finland, based on seed collected from young seed orchards with a low pollen production, indicate that pollination from southern sources is not as harmful to the adaptability of spruce as it is for pine seedlings (Ruotsalainen and Nikkanen 1998). Only a few studies have been carried out in Norway spruce seed orchards, in the conditions similar to ours, on flowering and pollination (Skrøppa and Tutturen 1985, Paule et al. 1993, Kjaer and Wellendorf 1997). In Finland, the studies of this thesis provide the first results dealing with the reproductive biology of spruce seed orchards, apart from the preliminary study on flowering (Ruotsalainen and Nikkanen 1989) performed using the same data (from 1984 to 1988) as in the thesis. Earlier studies in this field dealt with natural forests (Sarvas 1955, 1968, Koski 1970, 1971, 1973, Koski and Tallqvist 1978, Luomajoki 1993). In contrast, several comprehensive studies have been carried out in Scots pine seed orchards in Finland (Bhumibhamon 1978, Pulkkinen 1994a, Harju 1995).

The purpose of this thesis was to investigate how well the requirements of the functioning of an ideal seed orchard are fulfilled. In order to investigate this, various observations and measurements were performed in an operational seed orchard during the 15-year long study period. In addition, the purpose of the thesis was to estimate how the expected deviations from the ideal functioning affect the genetic composition of the seed produced in the orchard.

1.5 The objective of the thesis

The overall objective of the thesis was to study the functioning of a Norway spruce seed orchard and, in more detail, to investigate the clonal balance and synchrony of reproduction, and aspects of mating patterns and their effects on genetic diversity and quality of the seed crop.

The aims of the individual studies were:

I To describe the annual and clonal variation in flowering abundance, and to try to explain this variation on the basis of clonal and environmental factors. An additional aim was to estimate the genetic diversity of imaginary seed crops when clonal variation in female and male flowering and pollen contamination were considered.

II To determine the annual variation in the timing of flowering, and to describe the phenological variation in female receptivity and pollen dispersal. The aim was also to determine the extent to which genetic and environmental factors affect flowering phenology, and to discuss the consequences of reproductive phenology for the seed crop.

III To determine whether there is variation in pollen-tube growth among the seed orchard clones and, if such variation is found, whether it is connected with the characteristics of the pollen donors or any exogenous factors. The effect of different pollen germination conditions was also investigated.

IV To study pollen competition using controlled crossings with pollen mixtures including pairs of pollen lots with fast and slowly elongating pollen-tubes. Paternity analysis was performed in order to study whether the *in vitro* pollen germination vigour corresponds to the proportion of seeds sired by the pollen donor.

V To estimate the rate of pollen contamination and outcrossing in three different years, and in addition, in the thinned and unthinned parts of the orchard.

VI To investigate temporal and spatial variation in airborne pollen in the seed orchard and its immediate surroundings, and to estimate pollen contamination and self-fertilisation in different parts of the orchard. One specific aim was to analyse how the variation in airborne pollen affects mating patterns and quality characteristics of the seed.

Material and Methods

2.1 Basic information and management of the seed orchard

All the data for the studies of this thesis have been collected from Norway spruce seed orchard no. 170 (Heinämäki), established in 1968 at Korpilahti (62°13'N, 25°24'E). The seed orchard consists of 67 clones originating from latitudes 64°–67° N (Fig. 1, Nikkanen et al. 1999). Information about the seed orchard clones was obtained from the National Register of Forest Genetics.

The seed orchard is 13.2 ha in area, and is partly located on abandoned agricultural land (6.0 ha) and partly on forest land (7.2 ha) on a hill (160-190 m asl) sloping gently to the south and steeply to the east and west (Fig. 2, Fig. 2 in study **I**). The grafts were planted in the orchard using a clonal-row design with ramets of each clone in two or more rows distributed in different parts of the orchard. The spacing of the grafts was 3.5 x 6.5 m, the ramets of the same clone being located at a distance of 6.5 m from each other. In 1987 one half of the orchard was thinned systematically by removing every third graft, and in 1994 the other half of the orchard in the same way (Fig. 2 in **I**). The average number of ramets per clone was 56 before the first thinning, 47 after it, and 39 after the second thinning. The seed orchard was surrounded by spruce forest up until March 1994 when the closest part of the forest was felled (Fig. 2, Fig. in **VI**). Norway spruce is the predominant tree species in the region of the orchard.

The topography of the seed orchard and its immediate surroundings, and the position of the pollen samplers and the grafts, were mapped 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 whole study area (Lähde et al. 1992).

The nutrient concentrations and pH of the seed orchard soil were determined in 1993 (Hämäläinen 1994). In order to estimate the variation in the nutrient status, the seed orchard was divided into 20 plots. Plant-available phosphorus, potassium, calcium and magnesium were determined by extraction with 1N ammonium acetate (pH 4.65), and pH was measured on a soil sample/water suspension. The results of the soil analyses grouped into agricultural and forest land are shown in Table 1 in **I**.

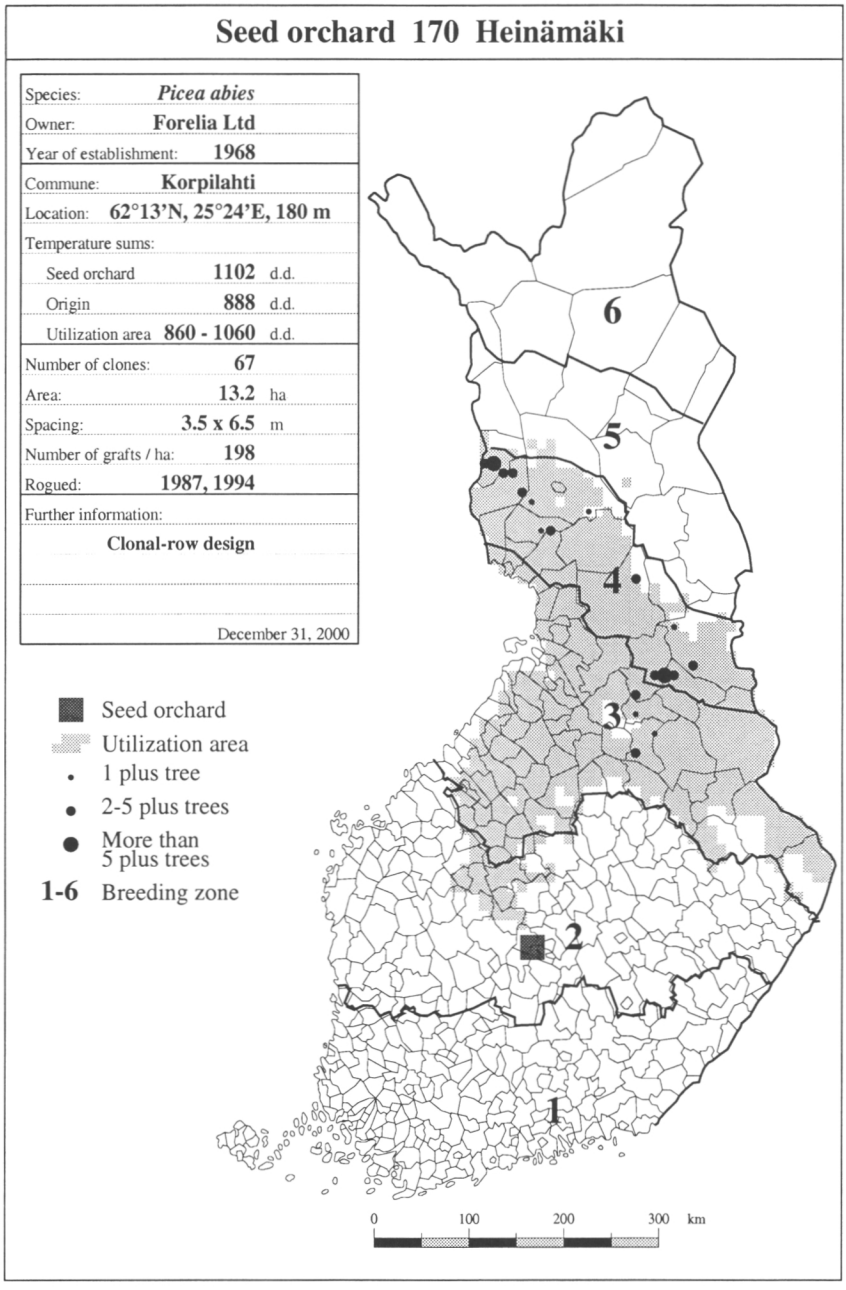

Figure 1. Location of the Heinämäki seed orchard and the origin of its clones, and the basic information about the seed orchard (Nikkanen et al. 1999).

Figure 2. Aerial view of the Heinämäki seed orchard. Photographed in March, 1995.

The height and diameter of the sample grafts (the same grafts used for measuring flowering abundance, see Chapter 2.3) were measured every year during 1984-1996 (except in 1994), and the width of the crown only once in 1993. The average height of the grafts in 1984 when the flowering study started was 4.9 m, the clonal means varying from 3.0 to 7.2 m. Twelve growing periods later in 1996 the average height was 10.4 m, varying from 6.5 to 13.4 m. Thus the average annual height growth of the grafts during the study period was 42 cm.

The seed orchard in many ways represents a typical or an average Norway spruce seed orchard in Finland. Its size and clone number are close to the average, and it has been planted using a rectangular spacing and clonal-row design, which is typical of two-thirds of all the orchards. In addition, this seed orchard also represents the five orchards (22%), with a total size of 87 ha (32%), established with northern origin and located in central parts Finland.

2.2 Weather data

The weather data were obtained both from the Jyväskylä weather station of the Finnish Meteorological Institute (62°24'N, 25°40'E, 140 m asl), located 25 km north-east from the seed orchard, and from the weather station (Datataker 610) set up in the orchard (Fig. 3). The weather data from Jyväskylä weather station consisted of annual, monthly and daily mean temperatures (including effective temperature sum, d.d., >+5°C) from 1982 to 1996 (Table 2 in **I**), as well as cloudiness and precipitation during the flowering period in some years (Table 1 in **II**). The data from our own weather station consisted of continuous temperature, illuminance, humidity, precipitation and wind speed and direction during the flowering period in 1995 (Table 1 in **VI**).

Figure 3. The observation tower with the weather station and pollen samplers in the Heinämäki seed orchard.

2.3 Measuring the abundance and phenology of flowering

Both female and male flowering were studied. The number of flowers was recorded on 66 of the 67 clones (one clone with two grafts was omitted from all the measurements) every year during 1984-1996 (**I**). In 1984 when the study was started, 10 sample grafts per clone were selected systematically to represent the whole seed orchard. After the thinning in 1987 the number of sample grafts was 5, but in 1988 it was returned to 10. No new sample grafts were subsequently selected. However, the thinning in 1994 and natural mortality had decreased the number of sample grafts to a minimum of 4 and average of 7 by 1996. The total number of grafts on which flowering abundance was measured varied from 650 to 478.

Pollen production was estimated on the basis of the number of male flowers on a graft. The amount of pollen produced by one male strobilus (0.009 g / strobilus, counted from 7041 strobili of 8 grafts in 1992) was used to calculate the amount of pollen produced by a graft.

The phenological stage of the female and male flowers was observed on seed orchard grafts in 1989, 1992, 1993 and 1995 (**II**). In 1989 the observations were made on 7 randomly chosen clones, in 1992 and 1993 on 21 randomly chosen clones with sufficient flowering abundance, and in 1995 on 65 of the 67 seed orchard clones. Observations on the phenological stage of the flowers were made on 3 grafts per clone.

2.4 Measuring the variation in airborne pollen

Temporal variation in airborne pollen was studied during a twelve-year period by means of a recording pollen sampler (Fig. 4, Sarvas 1962, 1968). The pollen catch was measured in the seed orchard from 1984 to 1995, and at a distance of 1 km to the southeast from the orchard from 1987 to 1995. The results obtained with the recording pollen sampler provided information about the actual timing of flowering in different years and about the daily fluctuation in airborne pollen during flowering (**II** and **VI**).

Spatial variation in airborne pollen was studied in 1995 using a rotorod type of sampler (Fig. 4, Edmonds 1972). A total of 70 samplers were situated on 48 masts, 1-3 samplers on each mast; 48 samplers 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 in **VI**). 37 samplers were located in the seed orchard and 33

outside it. A total of 24 ten-minute sampling periods were achieved during a seven-day period (Table 1 in **VI**). The rotorod-sampler gave an estimate of pollen density in the air. The pollen density values were interpolated for the whole study area by applying the spatial analysis methods described in study **VI**. Spatial variation in airborne pollen was described by means of density maps.

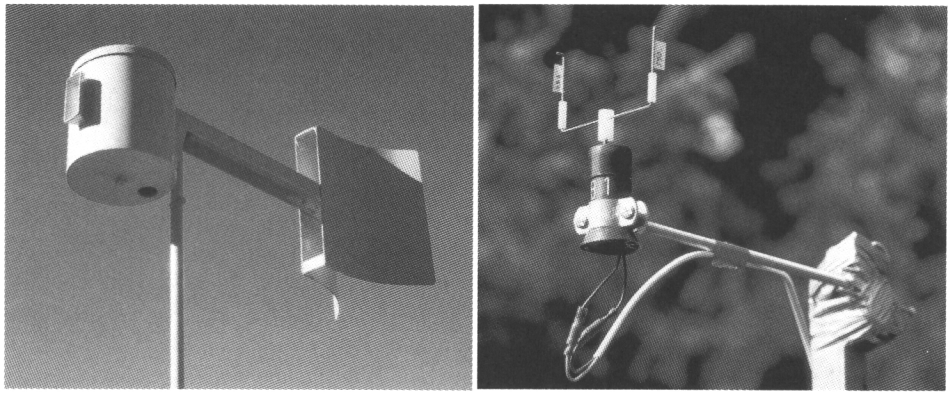

Figure 4. The pollen samplers used in the study: a) the Sarvas-Wilska type of recording pollen sampler, and b) rotorod type of pollen sampler.

2.5 Cone and seed sampling

Cones were collected in the seed orchard in 1989, 1992, 1993 and 1995, and on a smaller scale in 1998. The purpose of cone collection was to obtain material to investigate the variation in the quantity and quality of the seed crops in 1989 (Nikkanen 1992) and in 1995 (**VI**), and to obtain seed for isozyme studies on mating patterns in 1989, 1992, 1993 (**V**) and 1995 (**VI**), and for isozyme studies on pollen competition from controlled crossings in 1998 (**IV**).

The cone and seed crops were determined separately for each graft. 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).

2.6 Studying pollen competition *in vitro* and *in vivo*

Pollen samples were collected from 66 of the clones in the seed orchard in 1996 and 1998 in order to estimate pollen-tube growth rate in *in vitro* (**III**). One graft from each clone was selected as pollen donor (Fig. 1 in **III**). The same grafts were used in both years. In addition to the seed orchard clones, five trees from surrounding areas were used as pollen donors. More details about treatment of the pollen are given in study **III** and in Häggman et al. (1997). Germinated suspensions of pollen were photographed, and pollen-tube lengths were measured from the enlarged negatives. About 50 pollen grains were measured per replication, the total number of measurements being about 73 800.

Five pollen mixtures, based on differences *in vitro* germination vigour and with distinguishable isoentzyme genotypes (Table 1 in **IV**), were used to pollinate five seed parents (Table 2 in **IV**) in 1998. Each of the mixtures consisted of an equal mass of pollen from two clones, one assumed to have poor and the other good germination vigour. The selected pairs of pollen mixtures were used in controlled crossings to study pollen competition and seed-siring success.

2.7 Estimation of mating patterns

Seed for the isozyme studies was collected in 1989, 1992 and 1993 (**V**), and in 1995 (**VI**). Cones were collected from all the seed-producing clones of grafts representing different parts of the seed orchard. The multilocus genotypes of the embryos and haploid megagametophytes were assessed at 11 allozyme loci. More details about these loci and the technique used are given in studies **V** and **VI**, and in Muona et al. (1987). The pollen contamination, and outcrossing and self-fertilisation rates were estimated for the whole seed orchard, and separately for the different parts of the seed orchard. In addition, the rates of cross-fertilisation within the seed-orchard clones were estimated by summing up the estimates of pollen contamination and self-fertilisation

Paternity analysis, described by Smith and Adams (1983), was used in estimating pollen contamination. The multilocus genotypes of the pollen gametes were deduced by comparing the allozyme patterns in the megagamethophytes and in the corresponding embryos. Pollen genotypes that could not have been produced by any of the seed orchard clones were regarded as observed contamination (*b*). Because part of the contaminating pollen could not be distinguished from pollen produced in the orchard, the observed contamination had to be corrected by an estimate of

the detection probability of alien pollen (d), which was calculated using the gene frequencies of a local spruce stand and the gametes produced by orchard clones. The estimates of pollen contamination (m) were calculated as $m = b/d$. The formula for the variance estimate of contamination is given in Friedman and Adams (1985).

The multilocus method of Shaw et al. (1981) was used to estimate the rates of outcrossing (t), and selfing ($= 1\text{-}t$). 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 self-fertilisations, which was estimated by means of the gene frequencies of the seed orchard.

2.8 Estimation of genetic diversity

Genetic diversity of the seed orchard and the seed crops was described using the concept of effective number of clones (Kang and Lindgren 1999, Kang 2001, Kang et al. 2001), which was estimated using the method of status (effective) number (Lindgren et al. 1996, 1997, Lindgren and Mullin 1998). The effective number of clones (N_C) for the seed crop was calculated according to Lindgren and Mullin (1998) using the formula

$$N_C = \frac{1}{\sum_{i=1}^{n} p_i^2} \qquad (1),$$

where p_i can be any clonal proportion measured in the seed orchard. It is assumed that seed orchard clones are not related and have no inbreeding.

When the effect of pollen contamination was examined, the effective clone number was calculated according to Lindgren and Mullin (1998), and Ruotsalainen et al. (2000)

$$N_C = \frac{1}{\sum_{i=1}^{n} (f_i + (1-2M)m_i)^2} \qquad (2),$$

where f_i is the proportion of clone i of the female contribution (seed yield in this study) and m_i is the corresponding value for male contribution (pollen production). Both f_i and m_i sum up to 0.5. M is the proportion of migrating genes in the seed crop.

The effective number of clones was estimated for the seed orchard and for the imaginary seed crop, predicted on the basis of flowering, by adjusting stepwise for several sources of variation as described in **I**. In

addition, the effective clone numbers concerning the real seed crops of 1989 and 1995 were also estimated and presented in this thesis.

2.9 Statistical analyses

A non-parametric Kruskall-Wallis (**I**, **II**) test was used to determine the statistical differences between the clones, and the Spearman rank correlation procedure to calculate the strength of the linear association between different variables. A non-parametric test and rank correlation procedures were used, because the number of flowers had a skewed and non-normal distribution (**I**), and because the day scale used in the phenological observations was coarse (**II**).

When the restrictions mentioned above did not exist, analyses of variance were used to determine the statistical differences (**II**, **III** and **VI**), and the Pearson correlation procedure to determine the linear association (**III** and **VI**). The Tukey (**II** and **VI**) and Student-Newman-Keuls (**III**) post-hoc tests were performed for multiple comparisons of means.

Broad-sense heritabilities (h_B^2) were estimated on the basis of a single graft using the formula of Sokal and Rohlf (1995), as described in study **I** (and used also in **II**).

The Pearson χ^2-test was used to determine the differences in pollen contamination and outcrossing or selfing between years (**V**), and between different parts of the orchard (**V** and **VI**), as well as the significance of the paternal contribution (**IV**).

Linear stepwise regression analysis was used to obtain models to explain the variation in flowering abundance (**I**), and non-linear regression analysis to obtain a model to identify the parameters explaining the diurnal variation in the amount of airborne pollen (**VI**).

Results

3.1 Flowering abundance (I)

The between-year variation in both female and male flowering was large (Fig. 3 and Table 3 in **I**). In female flowering during the 13-year study period (from 1984 to 1996), there were 6 fairly abundant, 5 poor and 2 years with no flowering. Male flowering followed a similar pattern (Table 3 in **I**), and the estimates for pollen production in 1989, 1992, 1993 and 1995 were 11.2, 5.3, 4.8 and 23.4 kg/ha, respectively. Differences in flowering abundance among the clones were large and statistically significant. The average broad-sense heritability values for female and male flowering were 0.37 and 0.38, respectively, but varied considerably from year to year (Table 3 in **I**). The correlations between the flowering abundance of the clones in different years were usually positive and significant (Table 4 in **I**). However, there was also a strong tendency that, in two pairs of successive good flowering years, the same clones usually flowered well in the first year in both pairs of years, and the other clones in the second year (Fig. 4 in **I**). The clonal differences in flowering could not be explained by geographic origin, but were, excluding the second years (1993 and 1996), dependent on the graft size (Table 5 in **I**).

3.2 Reproductive phenology (II)

During the years examined (1985, 1986, 1987, 1989, 1992, 1993 and 1995), there were large differences in the onset of spring and in the weather during flowering (Table 1 and Fig. 2 in **II**). The duration of the receptive period of the seed orchard varied (in 1992, 1993 and 1995) from 5 to 8 days, and anthesis determined on the basis of airborne pollen (in the seven years above) from 5 to 10 days. The receptive period started normally about one day earlier than anthesis. In general, the flowering periods of the different clones overlapped (Fig. 3 in **II**). The clonal differences in the phenology of receptivity were in most cases statistically significant, but not in pollen shedding (Table 2 in **II**). The broad-sense heritability estimates were higher for female than for male phenology. Environmental factors, conversely, had a stronger effect on male phenology (Tables 4, 5 and 6 in **II**). Wide graft spacing and a graft position that favoured solar radiation on the lower parts of the crown promoted early pollen shedding.

3.3 Temporal and spatial variation in airborne pollen (II, VI)

The between-year variation in the timing of flowering was more than three weeks during the 7 years studied (Fig. 2 in **II**). The mean date of anthesis varied from May 15 to June 6, the average being May 28. The effective temperature sum of these dates varied from 122 to 159 d.d., the average being 141 d.d. Temporal variation in the amount of airborne pollen was large (Fig. 2 in **II**, Fig. 4 in **VI**).

In 1995 the duration of anthesis was 5 days (Fig. 3 in **II**, Fig. 4 in **VI**). The amount of airborne pollen increased during the first four days, and then decreased rapidly. Diurnal variation was high; the lowest amounts of pollen were measured at night and in early morning when, in general, the air humidity was high and wind speed low. During the first two days of anthesis, the pollen densities inside and outside the seed orchard were approximately the same, but from the third day onwards the densities in the orchard were higher (Fig. 5 and 6 in **VI**). 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 highest pollen density measured in the orchard was about 9000 pollen grains/m^3 of air (June 1, 4 p.m.), while the highest density outside and upwind side of the orchard was 3000 grains/m^3.

3.4 Pollen competition (III, IV)

Significant variation was found among the clones in the *in vitro* pollen-tube growth rate, the differences in the average pollen-tube lengths being 7- and 10- fold in different years (**III**). No correlation was found between the pollen-tube length and the phenology, growth or growing site characteristics of the pollen donors. However, there appeared to be pollen lots that either benefited from a higher germination temperature or germinated faster at lower temperatures (Fig. 2 in **III**).

When paternal success was studied using controlled crossings (**IV**), the success was found to be unequal: 15 of 23 crossings producing progeny differed significantly from the hypothetical ratio of 1:1 (Table 3 and 4 in **IV**). The paternal contribution in the majority of the crossings was as expected: the pollen parent with a more-vigorous *in vitro* germination sired more seeds than the parent with less-vigorous pollen (Table 3 in

IV). Despite aberrations in two of the pollen mixtures, the results support the hypothesis that pollen-tube competition is one of the factors contributing to male fitness in *Picea abies*.

3.5 Mating patterns (V, VI)

The mating patterns found in the seed orchard were far from the ideal. The estimated rates of pollen contamination in 1989, 1992 and 1993 were 0.69, 0.69 and 0.71, respectively (Table 1 in **V**). The contamination rate in the thinned parts of the orchard was significantly lower than that in the unthinned parts in two of the three years studied (Table 2 in **V**). The estimated outcrossing rate for the whole seed orchard was 0.96 in 1992 and 1.00 in 1989 and 1993 (Table 1 in **V**), indicating that the rate of self-fertilised seed produced in the orchard was negligible.

In 1995 the estimated rate of pollen contamination for the whole seed 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 (Fig. 1 and Table 4 in **VI**). The differences in the rate of contamination were significant between both the zones and the sections. The highest rate of contamination was estimated for the lowermost altitude zone and the lowest rate for the middle section of the orchard (Table 4 in **VI**). There were no significant differences in self-fertilisation between the zones or the sections, and the estimated rate of selfing in 1995 for the whole seed orchard was 0.06 (Table 4 in **VI**), which was clearly higher than that in 1989, 1992 and 1993 (in **V**). Significant negative correlation was found between the contamination and the accumulated amount of airborne pollen (Table 3 in **VI**).

The rate of cross-fertilisation within the orchard clones, after the rates of pollen contamination and self-fertilisation were summed up, was low. The cross-fertilisation rates in 1989, 1992, 1993 and 1995 were 0.31, 0.27, 0.29 and 0.23, respectively (**V** and **VI**). On the edges, in the lowest altitude zone of the orchard, the cross-fertilisation rate was only 0.06, and even in the middle section of the orchard it was not more than 0.45 (Fig. 1 and Table 4 in **VI**).

3.6 Genetic diversity and quality of the seed crop (I, VI)

The effective number of clones (status number) in the seed orchard was 56 when the variation in ramet number was considered. This is equivalent to 84% of the clone number (census number) of the orchard. The effective

clone numbers for imaginary seed crops after adjusting for the variation in both female and male flowering, and assuming equal ramet numbers for the clones, varied in different years from 23 to 55, the average being 39 (Table 7 in **I**). When the variation in ramet number was also considered the genetic diversity was lower, the effective clone number being on the average 32. Pollen contamination increased the effective clone number by about two-fold when the contamination rate (0.70) estimated in the orchard (**V** and **VI**) was considered (**I**).

The effective clone numbers for the imaginary seed crops in 1989 and 1995, after adjusting for the variation in ramet number and female flowering, were 30 and 25, respectively. The difference between the years was much larger after adjusting for cone production, and seed yield (Table 1) because of the cone and seed damage in 1995. In 1995 the average number of seeds extracted per cone and the proportion of full seed were low with high clonal variation (**VI**), while in 1989, when the quality of the seed crop was good, no lowering in the effective clone number was found (Table 1). Adjusting for the estimated pollen contamination together with the variation in pollen production increased the effective clone number, and thus the genetic diversity, considerably (Table 1).

Table 1. Estimated effective clone numbers (N_C) of the seed crop of the Heinämäki seed orchard in 1989 and 1995 after adjusting for different sources of variation in the genetic contribution of the clones and for pollen contamination.

Sources of variation	Effective clone number, N_C	
	1989	1995
Ramet number	56	56
Cone production	30	17
Cone production and seed yield	29	11
Above with pollen production and pollen contamination[1]	76	35

[1] 0.69 in 1989 and 0.71 in 1995

Discussion

4.1 Balance and synchrony of reproduction

Abundance of flowering

In *Picea abies* the annual variation in flowering abundance and cone crop is large (Blomqvist 1876, Heikinheimo 1932, 1948, Tirén 1935, Koski and Tallqvist 1978). During the 13-year study period the year-to-year variation in both female and male flowering in the seed orchard was large from year to year (Fig. 3 and Table 3 in **I**). The variation in female flowering could be explained reasonably well using Pukkala's (1987) model for predicting the seed crop on the basis of the temperature data of the two previous summers. The greatest incongruity between the prediction and the number of flowers in the seed orchard was observed when there was good flowering in two successive years (Fig. 3 in **I**).

The statistically significant and genetically determined variation in flowering abundance between the seed orchard clones in Heinämäki (Table 3 in **I**) is in accordance with earlier results for *Picea abies* in both natural stands and seed orchards (Sarvas 1968, Eriksson et al. 1973, Koski and Tallqvist 1978, Skrøppa and Tutturen 1985, Kjær 1996), as well as for other coniferous species (Varnell et al. 1967, Jonsson et al. 1976, Bhumibhamon 1978, Koski and Tallqvist 1978, Schoen et al. 1986, Matziris 1993). The correlations between the clones in different years were usually positive and significant (Table 4 in **I**). The finding that the correlation in female flowering between two successive good flowering years was poor (Table 4 in **I**) is in accordance with the results for *Picea abies* (Kjær 1996), *Picea glauca* (Schoen et al. 1986) and *Pinus nigra* (Matziris 1993). In the case of male flowering, the changes between successive years were not as great as those in female flowering, as also observed by Kjær (1996). The correlations for two pairs of successive good flowering years showed that there are genotypes with a different response to climatic factors: some clones flowered well in the first year, and other clones in the second year (Fig. 4 in **I**).

Differences in the origin of the clones did not explain the variation in flowering. The origins of the clones in the seed orchard may not have represented a sufficiently large area to show any clear differences in response to climatic adaptation. Instead, the clonal variation in flowering abundance was explained by the graft size, except in the case of female flowering for the latter years of the two successive good flowering years (Table 6 in **I**).

Reproductive phenology

The average date of flowering, measured as the mean date of anthesis, varied from May 15 to June 6 (Fig. 2 in **II**). The average temperature sum that had accumulated by these days was 141 d.d. (Table 1 in **II**), which was exactly the same temperature sum as Sarvas (1968) obtained for a natural stand in southern Finland, and close to the figure of 134 d.d. reported by Luomajoki (1993) for a natural stand located near the seed orchard. The timing of flowering was better determined by the temperature sum than the calendar day. The range of 37 d.d. (Table 1 in **II**) between the lowest and highest temperature sum values for the midpoint dates of anthesis corresponds to 6-7 days when calculated using the average daily temperatures during the flowering period. This is much smaller than the difference of 23 days observed in study **II**. The result is in accordance with the findings and conclusions of Sarvas (1968, 1972) and Koski (1991).

The duration of the receptive period in different years (1992, 1993 and 1995) varied in the studied seed orchard from 5 to 8 days, and that of anthesis (in 1985, 1986, 1987, 1989, 1992, 1993 and 1995) from 5 to 10 days (**II**). The receptive period started, at both the graft and clone level, slightly earlier than anthesis. In 1995 the receptive period and pollen shedding in the orchard occurred almost simultaneously, but in 1992 and 1993 pollen shedding was somewhat delayed (Fig. 3 in **II**). The short and rather simultaneous flowering period observed in this Norway spruce seed orchard is different from that reported for many other coniferous species especially in the temperate region (El-Kassaby et al. 1984, Griffin 1984, Askew 1988, El-Kassaby et al. 1988, Askew and Blush 1990, El-Kassaby and Reynolds 1990, El-Kassaby and Askew 1991, Matziris 1994). In a colder climate, shorter and more simultaneous flowering has been reported in seed orchards of *Pinus sylvestris* (Jonsson et al. 1976, Pulkkinen 1994b, Burczyk and Chalupka 1997) and *Picea mariana* (O'Reilly et al. 1982).

Although clonal differences in female and male flowering phenology were rather small, the ranking of the clones was in most cases similar from year to year (Table 3 in **II**). The correlation coefficients between female and male phenology were positive, but statistically significant only in one year. No results for *Picea abies* trees or clones showing correlation of flowering phenology between years or between female and male phenology within the same year have earlier been presented, but the phenomenon is well known in other coniferous species. In *Pinus sylvestris* the correlation in year-to-year flowering phenology among clones is positive and significant (Jonsson et al. 1976, Chung 1981a, Pulkkinen 1994b, Burczyk and Chalupka 1997). The order of the onset of receptivity and pollen shedding among clones also remains unchanged in

Pseudotsuga menziesii (El-Kassaby et al. 1984), *Pinus radiata* (Griffin 1984), *Pinus taeda* (Askew 1988), and *Pinus nigra* (Matziris 1994).

Female phenology was genetically more strongly determined than male phenology (Table 2 in **II**). The clonal differences at the start of receptivity were, in most cases, statistically significant but not in pollen shedding, and the broad-sense heritability estimates were higher for female than for male phenology. This can also be looked at in the opposite way: the environmental factors had a stronger effect on male than on female phenology. Very little attention has been paid to environmental factors in studies on reproductive phenology. The reason for this has been the relatively small variation between ramets compared to that between clones in many species and seed orchards (Jonsson et al. 1976, Wheeler 1983, El-Kassaby et al. 1984, Griffin 1984, Matziris 1994).

Airborne pollen

Temporal variation in the amount of airborne pollen (Fig. 2 in **II** and Fig. 4 in **VI**) could be explained on the basis of temperature sum, air humidity and wind speed (**VI**). The regular diurnal variation, i.e. a large amount of pollen during the day and a small amount at night, and its relationship with air humidity, has been observed among others by Sarvas (1955). However, if air humidity is relatively low at night, the amount of pollen in the air might be high even at that time. A good example of this is the night of May 31/June 1 in 1995 (Fig. 4 in **VI**) when the air humidity was lower than during the other nights, and there was sufficient wind. Sarvas (1962) reported a similar case in a natural *Pinus sylvestris* stand.

When the spatial variation in pollen density was investigated in detail in 1995 (Fig. 5 in **VI**), there was only slight variation during the first two days of anthesis, but the variation on the third and fourth day was much larger. The variation in pollen density in the seed orchard implies the effect of wind, and phenological differences in pollen shedding between the southern slope and the northern part of the orchard (**II**). The average pollen density increased in the seed orchard to a much higher level than outside it on the third day of anthesis (Fig. 6 in **VI**). This seems to indicate that the pollen caught in the orchard during the first two days was mainly derived from outside, but on the third day, because of the higher pollen densities, from the orchard. This is in accordance with the results (**II**) that abundant pollen shedding from the seed orchard grafts started on the third day of flowering, and was over within two days.

In 1995, as a result of the very warm weather (Table 1 in **VI**), the duration of flowering was short, and the time differences between the clones were small. However, the reproductive synchronisation was not complete even at that time. When pollen shedding in the orchard was at

its most abundant on the third and fourth day of flowering (Fig. 4 and 5 in **VI**), most of the female flowers in the orchard had been receptive for one to two days (Fig. 3 in **II**). In 1993, when flowering lasted longer, some of the 21 clones had passed their receptivity before the latest clones had started to shed pollen (**II**). This means that, in some years, some of the clones do not participate in the pollination of all the clones in the orchard.

The weight of one pollen grain of *Picea abies* is three times and its volume eight times greater than that of *Pinus sylvestris* (Lindgren et al. 1991). However, the pollen of both species has been found to be able to travel over long distances (Koski 1970, Lindgren et al. 1991). In addition to an ability to be distributed over long distances, pollen has to stay viable during the flight in order to be able to transfer genes. Pollen collected from the Heinämäki seed orchard remained viable in open paper bags in a greenhouse for two weeks (Fig. 3 in **III**). In another experiment, pollen exposed to direct ultraviolet radiation in an outdoor roof, stayed viable for several days (Lindgren and Lindgren 1996). Because pollen can travel for several hundreds of kilometres already during one day with a moderate wind speed, long-distance migration of functional pollen seems to be possible.

Pollen competition

In addition to clonal imbalance in flowering and seed production, and incomplete reproductive synchronisation, it has been suggested that pollen competition might be one of the reasons for unequal reproductive success also in conifers (Schoen and Cheliak 1987, Nakamura and Wheeler 1992, Skrøppa and Lindgren 1994). In study **III**, significant variation in pollen viability was found *in vitro* among the seed orchard clones. In addition, the pollen parent that germinated more vigorously *in vitro* sired more seeds than the less vigorous pollen when controlled crossings were performed with pollen lot mixtures that included fast- and slowly-elongating pollen tubes (**IV**). A similar connection between the pollen tube growth rate and parental success has previously been reported in a number of angiosperm species (Snow and Spira 1991, 1996, Pasonen et al. 1999).

The results presented here are in accordance with the reproductive biology of *Picea abies*, which provides an opportunity for male gametophyte competition. Sarvas (1968) calculated that the average capacity of a pollen chamber in *Picea abies* is 5 pollen grains. The significant variation in pollen viability, together with the observed genotype-environment interactions in pollen performance, may contribute to the variable genetic composition of the seed produced in the orchard. However, the influence of pollen competition among seed-orchard clones is not essential in

conditions where the rate of cross-fertilisation within an orchard is less than 0.30. On the other hand, if competition mainly takes place between orchard and non-orchard pollen, its effect on the genetic composition of the seed crop might be crucial.

Mating patterns

The basic factors in the mating patterns of seed orchards are cross-fertilisation within seed-orchard clones, self-fertilisation and pollen contamination (Adams and Birkes 1989). The primary aim of seed orchards is to ensure that all the mating occurs among the clones planted in the orchard, and that self-fertilisation and pollen contamination should be negligible (Sarvas 1970, Feilberg and Søegaard 1975). However, the estimated rates of cross-fertilisation in the Heinämäki seed orchard varied from 0.23 to 0.31 in the years studied (1989, 1992, 1993 and 1995). In 1995, when the mating patterns were estimated for the different parts of the orchard, the rate of cross-fertilisation was at its highest (0.45) in the middle, and at its lowest (0.06) on the edges of the orchard.

The estimated rates of self-fertilisation varied from year to year. In 1989 and 1993 the estimated rate of outcrossing was 1.00, and thus the rate of selfing, by definition (e.g. Brown et al. 1985), was 0.00. In 1992 when the rate of outcrossing was 0.96, the rate of selfing was 0.04 (**V**). In 1995 the selfing rate was 0.06 (**VI**). In 1989 and 1993, when no selfing was found in the orchard, the weather during flowering was colder and wetter than in 1992 and 1995 (Table 1 in **II**), and therefore the difference between the onset of the receptive period and pollen shedding was longer in 1989 and 1993 than in 1992 and 1995 (**II**). However, the selfing estimates for Heinämäki in 1992 and 1995 were lower than those estimated by Xie and Knowles (1994) for a Norway spruce seed orchard in Canada, but higher than the estimate of Paule et al. (1993) for two seed orchards in Sweden. The selfing rates estimated for Norway spruce stands have been higher than those for seed orchards (Müller 1977, Lundkvist 1979, Muona et al. 1990). Thus, the fear of injuriously high selfing rates in the clonal-row seed orchards, common in Norway spruce seed orchards in Finland, has been unnecessary.

The estimated rates of pollen contamination in the orchard were surprisingly similar, from 0.69 to 0.71 from year to year, in spite of the annual differences in weather conditions and timing of flowering (Table 1 and Fig. 2 in **II**), and in pollen production (from 5 to 23 kg / ha). The rates were also high when they are compared to the rates, 0.43 and 0.59, estimated by Paule et al. (1993) for two different seed orchards in Sweden. Although no differences were found in Heinämäki in the rate of

contamination between years, there were large and significant differences between different parts of the orchard (Table 2 in **V** and Table 4 in **VI**).

The high rates of pollen contamination, together with the results of temporal and spatial variation in airborne pollen, indicate that there is no phenological isolation between the seed orchard and the surrounding forests, as has been assumed (Sarvas 1970). However, when detailed sampling of airborne pollen was carried out inside and outside the orchard (**VI**), it was found that the pollen density inside the orchard increased considerably from the second to the third day of anthesis, but outside the orchard the greatest increase occurred one day later. This may indicate that there is some phenological difference in pollen shedding between seed orchard grafts and surrounding forests which, however, is too small to cause any phenological isolation. The possible effect of this phenological difference is, however, hidden under metandry (**II**), which is characteristic for both *Picea abies* and *Pinus sylvestris* (Sarvas 1968). According to Pulkkinen (1994b), this is pronounced in south-transferred Scots pine seed orchards, and one of the reasons for the high rates of contamination. Harju and Nikkanen (1996) have shown that, when pollination in Scots pine seed orchard is restricted to the pollination peak, pollen contamination is lower than that during the period of less abundant pollen shedding at the beginning of female receptivity. This also indicates that delayed pollen shedding from the seed orchard grafts could be one reason for the high pollen contamination.

One reason for the high rates of contamination may also be competition and selection during pollen tube growth or embryo development. In study **III** no difference in *in vitro* pollen-tube growth was found between seed orchard pollen and pollen from five trees outside the orchard. However, in a study on *Pinus sylvestris* using similar methods (Venäläinen et al. 1999), the pollen tube growth of clones from northern Finland was significantly slower than of those from southern Finland. Pulkkinen (unpublished data) has obtained similar results in Scots pine seed orchards.

4.2 Genetic diversity and quality of the seed crop

Genetic diversity

Study **I** demonstrated that the genetic diversity of the seed crop cannot be determined solely on the basis of the clone number of the seed orchard, but that variation in ramet number and flowering abundance, as well as pollen contamination, must be considered in order to estimate the effective number of clones for the seed-orchard crop (Table 7 in **I**). The relative effective clone number of the Heinämäki seed orchard, after

adjusting for the variation in ramet number, was 0.84, which is higher than the average (0.74) for Norway spruce seed orchards in Finland (Kang et al. 2001). When adjusting for the fertility variation of the orchard clones, the relative numbers obtained were on the average about 0.5, with large annual variation (Table 7 in **I**). The result that the fertility variation was smallest when flowering was the most abundant is in accordance with the findings of earlier studies on Norway spruce (Skrøppa and Tutturen 1985, Ruotsalainen and Nikkanen 1989). In addition, there are several stages during flowering to the seed crop that may affect the clonal contribution, and thus the genetic diversity of the seed crop (Sarvas 1968, Sweet 1975, Schoen et al. 1986, Schoen and Cheliak 1987). A considerable drop in the effective clone number due to variation in flowering and seed yield has been found in many conifer seed orchards (**I**, Kjaer and Wellendorf 1997, 1998, Gömöry et al. 2000).

According to the theory developed by Lindgren and Mullin (1998), and Ruotsalainen et al. (2000), pollen contamination will considerably increase the effective number of clones (Table 7 in **I**). The estimated rate of pollen contamination in the Heinämäki seed orchard in four different years (1989, 1992, 1993 and 1995) was about 0.7 (**V** and **VI**). With this rate of contamination the effective clone number of the seed crop, after adjusting for all the existing variation, would be about the same as the clone number of the orchard, and double the effective clone number without pollen contamination. The results show that the level of pollen contamination has a great effect on the genetic diversity of the seed orchard crop. In the calculations, pollen contamination was assumed to derive from an infinite population of unrelated trees. If the fertilising pollen grains are related to each other or to the seed orchard clones, then the effect of pollen contamination on the effective clone number will be smaller although still considerable (Lindgren and Mullin 1998).

In addition to clonal variation in flowering and cone production, variation in the ability to produce full seed may considerably lower the genetic diversity of the seed crop in years with poor crop quality. When the effective clone numbers in 1989 and 1995, after adjusting for the variation in cone production and seed yield, were examined (Table 1), it was found that the number was considerably lowered in 1995 (from 25 to 11) but not in 1989 (from 30 to 29). There were two reasons for the difference. After adjusting for the variation in female flowering (and ramet number), the difference in the numbers (30 and 25) was rather small, indicating higher fertility variation in 1995, but after adjusting for the variation in cone production, the number of 1995 decreased to 17. The reason for this decrease was underestimation of the number of flowers in abundantly flowering grafts in 1995 (data from **I** and **VI**). The number for the seed yield decreased even more to 11, which was due in this case to

cone and seed damage. Poor quality of the seed crop was reflected as the low average number of seeds extracted per cone (Table 2 in **VI**), but the low genetic diversity was caused by the large clonal variation in these traits.

In addition to differences in clonal contribution and pollen contamination, which gave rise to differences in effective clone numbers between years, there are sources of variation that do not necessarily appear in the number, but may considerably affect the gene frequencies of the seed crop. Even if the rate of pollen contamination would remain at the same level from year to year, the origin of migrating pollen may vary considerably between years in accordance with the prevailing weather and wind conditions. In years such as 1989 and 1993, when pollen shedding in the seed orchard, and probably also in surrounding forests, was more delayed compared to female receptivity in 1995 (**II**), the proportion of long-distance pollen might have been higher than that in 1995, even though the rate of contamination in the orchard was the same.

The patterns of pollination affect the quantity and quality of the seed-orchard crop. Poor pollination may lead to a decreased seed crop (Sarvas 1968, Harju and Nikkanen 1996) but, in addition, at least in *Picea abies*, to decreased genetic quality of seed crop, resulting from both increased inbreeding (Sarvas 1968, Koski 1970, 1973) and decreased genetic diversity due to more unbalanced flowering (**I**, Matziris 1993, Kjær 1996).

Quality of the seed crop

The quantity and quality of the seed crop can be decreased by a high rate of self-pollination or by insufficient pollination, or by damage that prevents normal development of flowers, or by rust and insect damage. Cone production in the Heinämäki seed orchard in 1995 was rather abundant, 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, but 87 in 1989 (Nikkanen, unpublished data). In addition, the proportion of full seed was lower in 1995 than in 1989. One reason for the low number of seeds per cone and for the low proportion of full seed may have been, in addition to rust and insect damage, a high rate of self-pollination, as indicated by the higher selfing rate of germinated seed in 1995 (Table 4 in **VI**) than in 1989, 1992 and 1993 (Table 1 in **V**).

In addition to large differences in the quantity and quality of the seed among the clones (**VI**), there were also large and significant differences between different parts of the orchard (Table 2 in **VI**). 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 (**I**). 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 the accumulated amount of pollen (Table 3 in **VI**), differences in the abundance of pollen may have affected this parameter.

4.3 Options to improve the functioning of seed orchards

Options in seed orchards at the seed-producing phase

The Heinämäki seed orchard, and the other Norway spruce seed orchards that are now at the seed-producing phase, are more than 30 years old. In 1989, after the long juvenile period, the orchards produced the first abundant seed crop (Nikkanen 1992), and since then there has been several abundant or moderate flowering years (**I**, Männyn, kuusen ja ... 1997). Although the spruce orchards have produced more seed than had been expected 10-15 years ago (Metsänjalostus- ja siemenhuolto-... 1992), there have also been problems, especially related to cone and seed damage, and irregular flowering. Evidently the irregularity of flowering has to be accepted, but the prevention of cone and seed damage is of crucial importance.

The management of Norway spruce seed orchards is limited to weed control and coppice cuttings and, in some of the orchards, fertilisation and cutting off the top of the crown of the grafts (topping) have also been carried out. So far, the Heinämäki seed orchard is the only spruce orchard that has been thinned. Thinning the spruce seed orchards has not been an urgent measure, because spruce grafts can tolerate shade without loosing the vitality of their lower branches, and because not enough progeny test results have been available for genetic thinning (Venäläinen 1993).

An ideal seed orchard has many targets and requirements, which are difficult to fulfil in an operational seed orchard. Deviations from the targets affect the genetic efficiency of the seed orchard. The studies of this thesis have confirmed that functioning of seed orchards is often far from ideal. There were large differences in flowering and seed production among the clones, and that pollen contamination was at a much higher level than expected when the orchard was established while, on the other hand, self-fertilisation was at a lower level. The assumption of random

mating between the clones was violated in Heinämäki by many factors: large fertility variation among the clones, incomplete reproductive synchronisation, and pollen competition.

The genetic efficiency of the seed orchards can be improved by adjusting for the mating patterns and clonal contribution. In seed orchards at the seed-producing phase, the most important management technique that can be used to affect both mating patterns (to minimise selfing and pollen contamination) and clonal contribution (to increase seed production and improve genetic gain) is thinning. Topping the grafts may have similar effects as thinning on mating patterns by increasing the amount of direct solar radiation reaching the lower parts of the crown. A number of other methods for decreasing pollen contamination in seed orchards in the open field have been tested, of which the most successful have been delaying the flowering of the grafts by cooling the flowers with water (Fashler and El-Kassaby 1987) and supplemental mass pollination (Wheeler 1983, El-Kassaby and Ritland 1986, Wheeler and Jech 1992, Eriksson et al. 1994). In addition, Lowe and Wheeler (1993) have suggested increasing pollen production by means of flower stimulation, and the creation of phenologically synchronous neighbourhoods within an orchard, as methods of decreasing the contamination. The only experience of these management methods in Finland concerns thinning and topping.

Thinning seed orchards has two different aims: to improve the growing conditions of the grafts, and to adjust for the contribution of clones (Werner 1975, Ilstedt 1982, Nikkanen and Pukkala 1986). Thinning can create more space, and subsequently result in more direct solar radiation to the grafts left in the orchard. In study **II** it was demonstrated that a wide graft spacing promotes early pollen shedding, which shortens the time difference between female receptivity and pollen shedding. It was also found (**V**) that, in some cases, pollen contamination in the thinned parts of the seed orchard was lower than that in the unthinned parts. All this advocates for keeping the orchard open enough to ensure more solar radiation and better ventilation for the lower parts of the crown. This then promotes early pollen shedding which, through the better reproductive synchronisation, will decrease pollen contamination.

The basic aim of thinning the Heinämäki seed orchard was to decrease the assumed probability of selfing. Because the orchard has been established using a clonal-row design, it was thinned systematically by removing every third graft from the clone row (Fig. 1 in **II**). However, as has been demonstrated in study **V**, thinning had no effect on the rate of selfing and, as has been already mentioned, the selfing rate remained at a reasonably low level compared to the results for other seed orchards and natural stands. However, the rate of 0.06 estimated in the orchard in 1995 (**VI**) can be considered as rather high for a seed orchard crop, because

every seed from the seed orchard is meant to be utilised (in forest cultivation). In natural forests, on the other hand, there is more room for natural selection.

When thinning is used to adjust clonal contribution it may have two, partly counteracting aims: to improve the genetic gain of the orchard by removing the clones with the worst breeding value, or to increase seed production by removing the clones with poor flowering and cone production. There are now results available from 15-year-old progeny tests for the genetic thinning of the Heinämäki seed orchard (Ruotsalainen and Nikkanen 1998), and there will soon also be results from 10-year-old tests for the other spruce orchards from the majority of the clones (Venäläinen 1993). In addition, clonal data from cone and seed production collected in 1989 (Nikkanen 1992) and 1995 (Nikkanen, unpublished data) from 12 seed orchards, including Heinämäki, are also available for use in genetic thinning.

A high level of pollen contamination is generally regarded as one of the greatest problems in conifer seed orchards (Lindgren 1991, Buchert 1992, Di-Giovanni and Joyce 1992, Wheeler and Jech 1992, Lowe and Wheeler 1993). Pollen contamination reduces the genetic gain obtained from seed orchards, and may reduce the adaptability of the seedlings (Lowe and Wheeler 1993). No results indicating a loss of genetic gain, due to contamination, are yet available from Norway spruce. In the case of Scots pine, non-orchard local pollination in south-transferred seed orchards has caused poor adaptability of seedlings when planted in the northern reforestation areas in Finland (Nikkanen 1982, Pulkkinen et al. 1995). The adaptability of Norway spruce seedlings has been found to be better in similar situations (Ruotsalainen and Nikkanen 1998).

Because the reduction of pollen contamination through seed orchard management methods has proved to be limited, other methods, applied outside the orchard, have also been tested. The nearest part of the spruce stand that closely surrounded the Heinämäki seed orchard in all directions, except in the northwest, was felled in 1994. After the felling, the nearest trees were about 40 meters from the orchard (Fig. 2 and Fig. 2 in **VI**). This clear-cutting was carried out in order to decrease the high level of pollen contamination estimated for the orchard (**V**). However, as was demonstrated in study **VI**, this had no effect; the contamination remained at the same level. Prior to felling, the trees around the orchard probably acted as a filter, and not only as pollinators. Ho (1992) has observed that the vegetation in the isolation zone will modify pollen flight and filter some pollen grains out before they reach the orchard. As the seed orchard stands on a hill without any shelter, it is now open and receptive to pollen clouds from every direction.

The genetic composition of the seed crop can still be adjusted during the cone collection and seed handling phases. Skrøppa and Tutturen (1985) suggested combining annual seed crops in order to equalise the clonal contribution of different years. The case of two pairs of successive good flowering years (**I**), when partly different clones flowered well in different years, is an example of the case where combining the crops will equalise the otherwise different crops. Annually similar or genetically desired seed crops can also be achieved by collecting cones clone-wise and mixing them at a later stage. The clone-wise collection of cones can also be used for adjusting the rate of selfing and pollen contamination, since large clonal differences in these traits have been found in many studies (Harju and Muona 1989, Savolainen and Kärkkäinen 1992, Savolainen et al. 1992, Xie and Knowles 1994). In clone-wise collection, or in thinning, the clones with a high rate of selfing or contamination can be left out.

Selective cone collection can also be directed spatially, for instance, by omitting the fringes of the orchard in collection. Since there were large differences in pollen contamination between different parts of the orchard (**VI**), this is one way of decreasing the rate of contamination in the crop. However, a relatively large part of the orchard (about one-third) had to be omitted from the collection in Heinämäki in order to decrease the rate of contamination by 10 or 20%, and even then the contamination still remained at the level of 50%.

Options in planning new seed orchards

There are more options to improve the genetic efficiency of seed orchards when new orchards are being planned than when old orchards are to be improved. In the late 1990's, after a break of 25 years, two (total area 12 ha) new Norway spruce seed orchards were established (National Register of Forest Genetics), and at least 100 hectares of new spruce seed orchards are planned to be established before 2020 (Männyn, kuusen ja ... 1997). All the information available about the breeding value and production ability of the clones, as well as knowledge of the factors influencing their functioning, have to be taken into consideration when planning new orchards.

The studies of this thesis have raised some ideas about the general structure and genetic composition of an effective seed orchard. The basic question is, shall we try to avoid pollen contamination or shall we accept it. This will affect many of the traits of a new generation seed orchard. If the option of avoiding contamination is selected, there are several factors that need to be considered. First of all the orchard has to be large enough (at least 20 ha in area), and it has to be located in areas where spruce forests are rare. However, because Norway spruce is rather common

throughout Finland, and because its pollen is able to fly over long distances, it is impossible to completely avoid contamination anywhere in the country. In order to minimise contamination other methods, like increasing pollen production and promoting early pollen shedding, have also to be utilised to promote cross-fertilisation within the orchard clones. However, probably the only effective methods of avoiding contamination are to establish the seed orchard in areas with phenological or geographical isolation (Koski 1987, Lowe and Wheeler 1993) or to establish it under a plastic cover (Mikola 1993a). When applying these methods, which drastically change the conditions during the flowering period, we may, however, be faced with physiological after-effects that affect the growth rhythm of seedlings originating from the orchards (Johnsen 1988, 1989, Skrøppa et al. 1994, Johnsen et al. 1995, Skrøppa and Johnsen 2000).

In the case of the other option, when pollen contamination is accepted and, more over, when it is opted for the part of the strategy to produce genetically improved seed, the optimum structure, and also the genetic composition of the seed orchard will be different from the one described above. On the contrary to the first option, in this one the orchard has to be located close to spruce forests, and it can be small in size or of such a shape that background pollination is promoted. When the aim is not to promote pollen production or early pollen shedding, the orchards can be established using a denser spacing than was used in the old orchards, also in order to utilise them effectively at young age. In addition, the clones producing a lot of female flowers but not as many male flowers can be favoured.

When new effective seed orchards are planned, genetic diversity and genetic gain have to be combined. According to the theory, the complete pollination with non-orchard pollen increases 4-fold the effective number of clones compared to the situation for complete panmictic cross-fertilisation within the orchard clones (Lindgren and Mullin 1998). Thus, in order to achieve the same genetic diversity in the seed crop as in the ideal traditional seed orchard, only one-fourth of the number of clones is needed. The lower clone number enables more effective selection of clones with a higher breeding value, and this will probably compensate for the loss of genetic gain due to pollen contamination. In this option, several orchards with a small size and low clone number are established, instead of one of the traditional type of seed orchard.

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I

Erratum

Explanations for formula 1 on page 209 should be:

where K_1 and K_2 are the mean temperature indices (the mean temperatures are compared to corresponding long term averages and expressed as percentages) of June one and two years before flowering, respectively, and H_1 and H_2 are the respective mean temperature indices of July.

Variation in Flowering Abundance and Its Impact on the Genetic Diversity of the Seed Crop in a Norway Spruce Seed Orchard

Teijo Nikkanen and Seppo Ruotsalainen

Nikkanen, T. & Ruotsalainen, S. 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34(3): 205–222.

The variation in flowering abundance was studied in a Norway spruce seed orchard, located in southern Finland (62°13'N, 25°24'E), consisting of 67 clones from northern Finland (64°–67°N). The flowering variation in 1984–1996 was studied at the annual, clonal and graft level. In addition, the genetic diversity of an imaginary seed crop was estimated using a concept of status number.

The between-year variation was large in both female and male flowering. Differences in flowering abundance among the clones were large and statistically significant in all the years studied. The average broad-sense heritability values for female and male flowering were 0.37 and 0.38, respectively, but varied considerably from year to year. The correlations between the flowering abundance of the clones in different years were usually positive and significant. However, the correlations for two pairs of successive good flowering years showed that the same clones usually flowered well in the first year in both pairs of years, and the other clones in the second year. The clonal differences in flowering could not be explained by geographic origin, but were more dependent on the graft size. Our results demonstrate that the variation in the ramet number, flowering abundance and pollen contamination must be included when estimating the genetic diversity of the seed crop in a seed orchard. The relative status number of the seed orchard was 84% of the number of clones when the variation in the ramet number was included. The relative status numbers after adjusting for the variation in female and male flowering were on the average 46 and 55%, respectively, and 59% when adjusting for both genders together. Pollen contamination increased the status number considerably.

Keywords *Picea abies*, clone, ramet, status number, census number
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1 Introduction

The results of forest tree breeding are utilised through artificial regeneration. Most of the seed for artificial regeneration in Finland is nowadays produced in clonal seed orchards consisting of genetically superior trees. Because the number of seed orchards, number of clones per seed orchard and total number of clones are rather limited in Norway spruce (*Picea abies* (L) Karst.) seed orchards (Nikkanen et al. 1999) especially, it is important to pay attention to the genetic diversity of the regeneration material produced in seed orchards.

Variation in flowering affects the genetic diversity of the seed crop. The between-year variation in the abundance of flowering and seed crop of Norway spruce is large (Blomqvist 1876, Heikinheimo 1932, 1948, Tirén 1935, Koski and Tallqvist 1978). The periodicity of abundant flowering is irregular, and the occurrence of good flowering years is the more infrequent, the more northern is the region in question (Koski and Tallqvist 1978). The annual variation in flowering can primarily be explained by climatic factors. High temperatures during the differentiation of flower buds promote abundant flowering (Lindgren et al. 1977, Pukkala 1987). The weather in early and mid-summer is crucial, because the differentiation of flower primordia takes place not later than July. The seed crop of Norway spruce can be predicted rather well on the basis of the June and July temperatures in the two preceding summers (Pukkala 1987).

The variation in flowering abundance is also large between trees within a stand (Sarvas 1968, Koski and Tallqvist 1978), and between clones within a seed orchard (Skrøppa and Tutturen 1985, Ruotsalainen and Nikkanen 1989, Kjær 1996). When Norway spruce is planted in a locality where the summer temperatures are higher than those to which it is genetically adapted, it responds by enhancing female flowering (Skrøppa and Tutturen 1985). In Finland, as well as in the other Nordic countries, Norway spruce and Scots pine (*Pinus sylvestris* (L.)) seed orchards have often been established at sites with a warmer climate than that from which the selected plus trees originate, and where the orchard seed is to be used (Sarvas 1970, Werner 1975, Ilstedt and Eriksson 1982, Skrøppa and Tutturen 1985).

For estimating genetic diversity in natural populations, Wright (1931) introduced the concept of effective population size. Since then, the concept has been developed and applied by many population geneticists and plant breeders, mainly focusing on two alternative aspects; the inbreeding effective population number and the variance effective population number (Crow and Kimura 1970, Crow and Denniston 1988, Muona and Harju 1989, Caballero 1994, Burczyk 1996, Kjær 1996). Because effective population size describes the rate of change in a population, Lindgren et al. (1996, 1997) developed the concept of status number, which is a more functional measure of the state for a non-changing population, like a seed orchard crop. The application of status number for estimating the genetic diversity of seed orchards or seed orchard crops has been discussed by Kjær and Wellendorf (1997, 1998), Lindgren and Mullin (1998), Kang and Lindgren (1998) and Ruotsalainen et al. (2000), and is continued in the present work.

Various aspects of flowering and seed crop have been intensively studied in a Norway spruce seed orchard, Heinämäki, in southern Finland (Ruotsalainen and Nikkanen 1989, Nikkanen 1993, 1995, 2000, Puhakka 1993, Hämäläinen 1994, Pakkanen et al. 2000). This seed orchard was selected for the study in 1983 because it was one of the first Norway spruce seed orchards to start reasonable flowering. It also well represents the specific problems encountered in Finnish Norway spruce seed orchards: clonal-row design, pollen contamination and transfer of clones from north to south. Due to the long time series of flowering this seed orchard offered excellent material for the present study.

The aim of this study was to determine the magnitude and characteristics of flowering variation in a Norway spruce seed orchard, and to try to explain the variation on the basis of clonal and environmental factors. The variation in flowering abundance and pollen contamination was used to estimate their effect on the genetic diversity of the seed crop produced in the seed orchard.

2 Material and Methods

2.1 Basic Information and Management of the Seed Orchard

The variation in flowering abundance was 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 (Fig. 1). The effective temperature sum (+5 °C threshold) of the seed orchard location was 1100 d.d., and that of the plus tree locations varied from 820 to 1070 d.d. (Nikkanen et al. 1999).

Information about the geographic origin of the clones was obtained from the National Register of forest genetics. The geographic data were used to calculate predicted climatic variables for the original growing sites of the plus trees. This was done by a program (ILMA) that interpolates climatic variables to any location in Finland using the measurements made at weather stations (Ojansuu and Henttonen 1983). The original geographic data and climatic variables derived from it were used to explain the clonal variation in flowering.

The seed orchard is 13.2 ha in area, and is partly located on abandoned agricultural land (6.0 ha) and partly on forest land (7.2 ha) on a hill (160–190 m asl) sloping gently to the south and steeply to the east and west (Fig. 2). The grafts were planted in the orchard using a clonal-row design with ramets of each clone in two or more rows distributed in different parts of the orchard. The spacing of the grafts was 3.5 × 6.5 m, the ramets of the same clone being located 6.5 metres from each other. In 1987 one half of the orchard was thinned systematically by removing every third graft, and in 1994 the other half of the orchard in the same way (Fig. 2). The average number of ramets per clone was 56 before the first thinning, 47 after it, and 39 after the second thinning. In the early part of the study period the seed orchard was surrounded by old Norway spruce forest which was cut down in winter 1994.

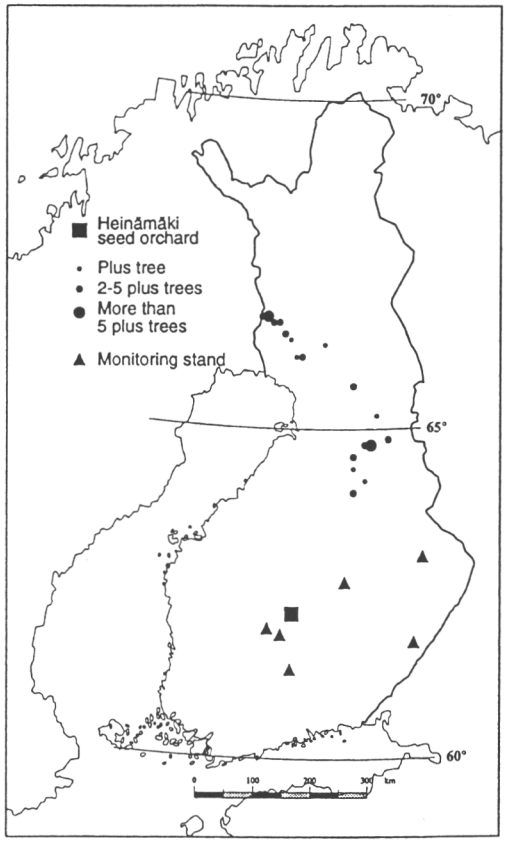

Fig. 1. Location of the Heinämäki seed orchard and the origin of its clones, and the monitoring stands for flowering abundance.

2.2 Soil and Weather Data of the Seed Orchard

The nutrient concentrations and pH of the seed orchard soil were determined in 1993 (Hämäläinen 1994). In order to estimate the variation in the nutrient status, the seed orchard was divided into 20 plots and soil samples were taken down to a depth of 5–15 cm at 20 points on each plot. The samples were bulked to give one composite sample per plot. Plant-available phosphorus, potassium, calcium and magnesium were determined by extraction with 1N ammonium acetate (pH 4.65), and pH on a soil sample/water suspension. The results were used as independent

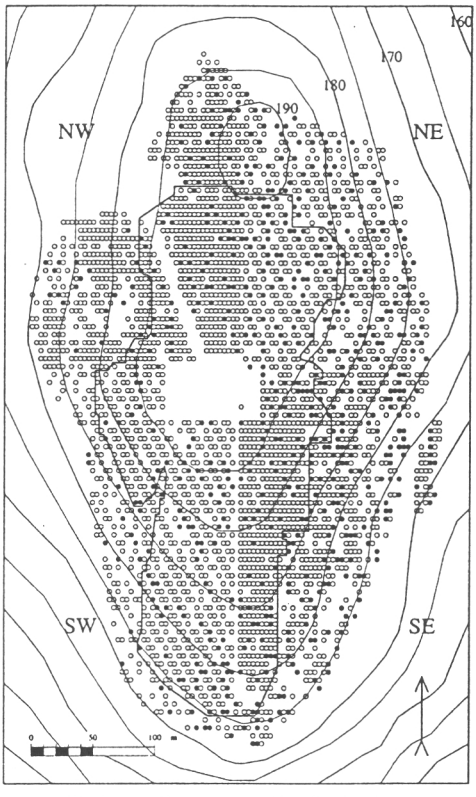

Fig. 2. The Heinämäki seed orchard in 1993. The orchard is situated on a hill and divided into four sections (NW, NE, SW and SE). Two of the sections (NE and SW) were thinned systematically in 1987, and the rest of the orchard in 1994. The border line between the abandoned agricultural (in the middle) and forest land, and the altitude contours are marked on the map. The sample grafts observed after 1988 are also marked.

variables to describe the differences in flowering abundance or graft size. The results of the soil analyses grouped into agricultural and forest land are shown in Table 1.

The weather data for the study period were obtained from the Jyväskylä weather station of the Finnish Meteorological Institute, located 25 km north-east from the seed orchard. The weather data consisted of annual, monthly and daily mean temperatures (including effective temperature sum, d.d.) from 1982 to 1996. The annual mean temperatures and temperature sums, and some important monthly mean temperatures are shown in Table 2.

Table 1. The concentrations of plant available phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) and pH (soil/water) in the soil of the Heinämäki seed orchard in 1993.

Nutrient	Agricultural land		Forest land		Total	
	$\bar{x}$	CV %	$\bar{x}$	CV %	$\bar{x}$	CV %
P mg/l	1.9	49.1	1.7	50.1	1.8	50.2
K mg/l	64.0	19.4	60.3	26.7	62.3	23.1
Ca mg/l	680.9	23.4	360.3	42.4	527.6	42.4
Mg mg/l	34.6	27.4	34.4	50.6	34.5	40.1
pH	5.8	2.8	5.4	1.9	5.6	4.3

Table 2. The annual effective temperature sums and mean temperatures of the study period recorded at the Jyväskylä Weather Station of the Finnish Meteorological Institute.

Year	Temperature sum (> +5 °C)	Mean temperature				
		Annual	May	June	July	August
	d.d.		°C			
1982	999	2.9	8.1	10.0	16.5	14.2
1983	1220	3.4	10.6	13.4	16.6	13.5
1984	1211	3.6	12.3	12.8	15.0	13.2
1985	1141	0.8	7.7	13.3	15.3	14.8
1986	1154	2.5	10.2	16.4	16.3	12.1
1987	892	0.6	7.0	12.7	14.2	10.5
1988	1331	3.0	9.5	15.8	19.2	13.2
1989	1254	4.7	9.9	15.6	15.8	13.4
1990	1059	3.9	8.4	13.1	14.7	14.2
1991	1086	3.7	6.0	12.5	16.5	15.0
1992	1176	3.6	10.4	15.2	13.3	12.7
1993	994	2.9	11.6	10.6	15.1	12.5
1994	1143	2.8	6.6	12.5	18.2	14.1
1995	1263	3.7	8.2	16.5	14.7	14.2
1996	1070	2.6	7.7	13.0	13.8	16.1
Average in 1982–96	1133	3.0	9.0	13.6	15.7	13.6
Average in 1961–90	1129	2.6	8.7	14.1	15.7	13.6

2.3 Measuring Flowering Abundance and Size of the Grafts

Both female and male flowering were studied. The number of flowers was recorded every year during 1984–1996, the observations being made during flowering in May and June. The number of male flowers on sample branches was counted and used to evaluate the total number of male flowers on a graft. In 1984 when the study was started, 10 sample grafts per clone were selected systematically to cover representatively the whole seed orchard. After the first thinning in 1987 the number of sample grafts was 5, but in 1988 it was returned to 10 using systematic selection. No changes in the number of sample grafts were subsequently made. However, the second thinning in 1994 and natural mortality decreased the number of sample grafts to a minimum of 4 and average of 7 in 1996. The total number of grafts on which flowering abundance was measured varied from 650 to 478.

The height and diameter of the sample grafts were measured in all years except 1994. The width of the crown was measured in 1993. The average height of the grafts in 1984 when the flowering study started was 4.9 m, the clonal means varying from 3.0 to 7.2 m. Twelve growing periods later in 1996 the average height was 10.4 m, varying from 6.5 to 13.4 m. Thus the average annual height growth of the grafts during the study period was 42 cm. The size of the graft was used as one of the independent variables to describe flowering abundance.

The flowering abundance in natural stands, used as comparisons when the annual variation in flowering was studied, was obtained from 6 monitoring stands in different parts of southern and central Finland (Fig. 1) (Hokkanen, unpublished data).

2.4 Data Analysis

The annual variation in female flowering was explained using the model for predicting a seed crop developed by Pukkala (1987). The formula predicting the seed crop (*SI*) in southern Finland (Pukkala 1987, p. 138; corrected for a printing error) is

$$SI = 175.35 + 0.05144\left(\frac{K_1 + H_1}{2}\right)^2 - 5.9860\left(\frac{K_2 + 2H_2}{3}\right) \quad (1)$$

where K_1 and K_2 are the mean temperatures of June one and two years before flowering, respectively, and H_1 and H_2 are the respective mean temperatures of July.

The variables measured on the grafts in the seed orchard were used in the statistical analyses as such, as well as after some transformations. In order to describe the changes in flowering between successive years new variables were created by subtracting the number of flowers in one year from that in the following year.

Because the number of flowers had a skewed and non-normal distribution, a non-parametric Kruskall-Wallis test was used to test the statistical differences between the clones. For the same reason the Spearman rank correlation procedure was used to calculate the strength of the linear association between different variables. Stepwise regression analysis was used to obtain models that best explained the variation in flowering. The variance components for estimating heritability values were obtained by analysis of variance. All these analyses were performed by SPSS® Base 8.0 statistical software (SPSS Inc. 1998).

Broad-sense heritabilities (h_B^2) (= clonal repeatability) were determined on a single-graft basis for each study year separately using formula (2) (Sokal and Rohlf 1995). The procedure is similar to that described in Matziris (1993). Standard errors for the estimates were determined using the approximate formula given by Becker (1984).

$$h_B^2 = \frac{s_c^2}{s_c^2 + s_e^2} \quad (2)$$

where s_c^2 is the variance component for clonal differences, and s_e^2 is the environmental variance.

Genetic diversity of the seed orchard and the seed crops was described using the status number (N_S), which is derived from group coancestry (Θ) (Lindgren et al. 1996, 1997, Lindgren and

Mullin 1998). The status number for the seed orchard crop was calculated according to Lindgren and Mullin (1998) using formula (3), which assumes that the seed orchard clones are not related to each other and have no inbreeding.

$$N_S = \frac{1}{\sum_{i=1}^{n} p_i^2} \quad (3)$$

where p_i can be any clonal proportion measured in the seed orchard (female or male flowering, graft proportion).

When the effect of pollen contamination was considered, the status number was calculated according to Lindgren and Mullin (1998), and Ruotsalainen et al. (2000)

$$N_S = \frac{1}{\sum_{i=1}^{n} \left(f_i + (1-2M)m_i\right)^2} \quad (4)$$

where f_i is the proportion of clone i of the female flowers and m_i is the corresponding value for male flowering. Both f_i and m_i sum up to 0.5. M is the proportion of migrating genes in the seed crop. Here also the clones were assumed to have no relatedness and no inbreeding. A further assumption was that the contaminating pollen is not related to itself or to the seed orchard clones.

The status number for combined proportion of female and male flowering ($c_i = f_i + m_i$) was also weighted with the graft proportions (g_i). The weighted proportions were calculated using formula (5)

$$p_i = \frac{g_i c_i}{\sum g_i c_i} \quad (5)$$

The status numbers were estimated for the seed orchard and the seed crops adjusting for several sources of variation. The first factor to be considered was the variation in ramet number. In this adjustment the clonal ramet contributions (p_i) were inserted in formula (3), it being assumed that there are no clonal differences in flowering abundance. The next step was to assume an equal number of ramets per clone, and to study the effect of variation in flowering abundance on status number. This was done separately for female and male flowering, and also for the combined flower contribution. When the variation in female or male flowering was studied separately, it was assumed that the contribution of the other sex was the same as that of the studied one. The study was brought closer to the real situation when both the variation in ramet numbers and flowering abundance were combined using formulae (5) and (3). Finally the status numbers for the seed crop were estimated by considering the variation in ramet numbers and flowering abundance, and assuming different levels of pollen contamination (formula 4). The rationale in this kind of stepwise approach is that it shows the possibilities of utilising different levels of information about the genetic contribution of the clones in estimating the genetic diversity of the seed crop.

3 Results

3.1 Annual Variation in Flowering

The year-to-year variation in flowering was large in both female and male flowering (Table 3). During the 13-year study period there were six years (1987, 1989, 1992, 1993, 1995 and 1996) when flowering was fairly abundant, five years (1984, 1985, 1986, 1990 and 1991) when it was poor, and two years (1988 and 1994) when there was no flowering in the orchard.

Flowering was the most abundant in 1996, when the average number of female flowers per graft was 87 and male flowers 17 300. The maximum number of female flowers per single graft was 680 and of male flowers 100 000. However, the percentages of flowering grafts were greater in 1989 and 1993 than in 1996 (Table 3).

The model predicting the annual seed crop (formula 1) on the basis of the temperature data of two previous summers gave a rather good fit ($r = 0.71$, $p = 0.007$) when the formula for southern Finland was used (Fig. 3). The formula for northern Finland (Pukkala 1987) gave a poorer prediction ($r = 0.41$, $p = 0.163$).

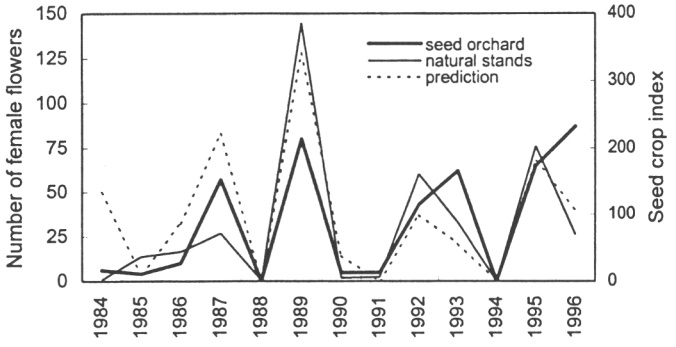

Fig. 3. Annual variation in the number of female flowers in the Heinämäki seed orchard and in 6 natural stands in southern and central Finland, and the predicted seed crop using Pukkala's (1987) model.

Table 3. The percentages of flowering grafts, clonal mean, minimum and maximum values for the number of flowers, and the broad-sense heritability values for the number of flowers in the Heinämäki seed orchard in different years.

Year	Percentage of flowering grafts		Number of female flowers / graft			Number of male flowers / graft			Broad sense heritability	
	Female	Male	Mean	Min	Max	Mean	Min	Max	Female	Male
1984	36	59	6	0	33	310	0	1770	0.19	0.30
1985	31	48	4	0	39	530	0	6390	0.44	0.41
1986	44	88	10	0	127	1080	0	4950	0.37	0.33
1987	77	91	57	0	196	1700	10	7300	0.42	0.49
1988	0	0	0	0	1	90	0	980		
1989	96	99	80	0	454	4900	73	16950	0.27	0.35
1990	54	91	5	0	18	550	4	2010	0.17	0.46
1991	31	66	5	0	20	620	0	2570	0.36	0.39
1992	90	83	43	0	278	2190	0	6880	0.39	0.39
1993	95	99	62	0	202	2080	184	5230	0.42	0.40
1994	0	0	0	0	0	0	0	1		
1995	82	83	65	0	396	12500	0	47200	0.46	0.40
1996	79	98	87	0	496	17300	1207	65000	0.63	0.30
Average	55	70	33			3373			0.37	0.38
sd	33	33	32			5158			0.12	0.06

3.2 Clonal Differences in Flowering Abundance

Differences in flowering abundance among the clones were large (Table 3) and statistically significant (p 0.000) in all the years studied. The proportion of clones that did not flower at all was largest when flowering was poor. In the years of abundant flowering all the clones in the seed orchard flowered. For female flowering such years were 1989, 1992, 1993 and 1996, and for male flowering 1987, 1989, 1990, 1993 and 1996. Broad-sense heritability estimates for flowering abundance varied considerably from year to year (Table 3). The average heritability estimates for female and male flowering were slightly higher

Table 4. The Spearman rank correlation coefficients of the clones (significance in parentheses) between female and male flowering in different years (diagonal), and between years in female (above diagonal) and male flowering (below diagonal) in the Heinämäki seed orchard.

Year		1987	1989	1992	1993	1995	1996
		Female					
1987		0.58 (0.000)	0.49 (0.000)	0.48 (0.000)	0.55 (0.000)	0.32 (0.009)	0.44 (0.000)
1989		0.72 (0.00)	0.39 (0.001)	0.74 (0.000)	0.51 (0.000)	0.38 (0.002)	0.39 (0.001)
1992	Male	0.70 (0.000)	0.85 (0.000)	0.56 (0.000)	0.30 (0.013)	0.64 (0.000)	0.23 (0.068)
1993		0.52 (0.000)	0.65 (0.000)	0.55 (0.000)	0.17 (0.182)	–0.01 (0.962)	0.73 (0.000)
1995		0.60 (0.000)	0.79 (0.000)	0.84 (0.000)	0.56 (0.000)	0.56 (0.000)	–0.17 (0.161)
1996		0.18 (0.148)	0.27 (0.030)	0.21 (0.092)	0.47 (0.000)	0.08 (0.530)	0.38 (0.001)

Table 5. The Spearman rank correlation coefficients (significance in parentheses) between female and male flowering, and origin (latitude) of the clones and size (height, breast height diameter and crown volume) of the grafts in the Heinämäki seed orchard.

Year	Latitude ×		Height ×		Diameter ×		Crown volume ×	
	Female flowering	Male flowering	Female flowering	Male flowering	Female flowering	Male flowering	Female flowering	Male flowering
1987	0.21 (0.097)	–0.11 (0.361)	0.37 (0.002)	0.57 (0.000)	0.33 (0.007)	0.60 (0.000)	0.36 (0.003)	0.46 (0.000)
1989	0.07 (0.577)	–0.26 (0.037)	0.59 (0.000)	0.50 (0.000)	0.60 (0.000)	0.55 (0.000)	0.64 (0.000)	0.49 (0.000)
1992	–0.13 (0.308)	–0.21 (0.095)	0.55 (0.000)	0.56 (0.000)	0.50 (0.000)	0.63 (0.000)	0.54 (0.000)	0.56 (0.000)
1993	0.12 (0.358)	–0.28 (0.022)	0.19 (0.130)	0.42 (0.001)	0.16 (0.197)	0.47 (0.000)	0.34 (0.006)	0.27 (0.026)
1995	–0.16 (0.199)	–0.33 (0.006)	0.38 (0.002)	0.48 (0.000)	0.39 (0.001)	0.51 (0.000)	0.32 (0.009)	0.42 (0.000)
1996	0.17 (0.176)	–0.07 (0.598)	0.001 (0.994)	0.17 (0.180)	–0.09 (0.478)	0.33 (0.006)	0.10 (0.422)	0.19 (0.124)

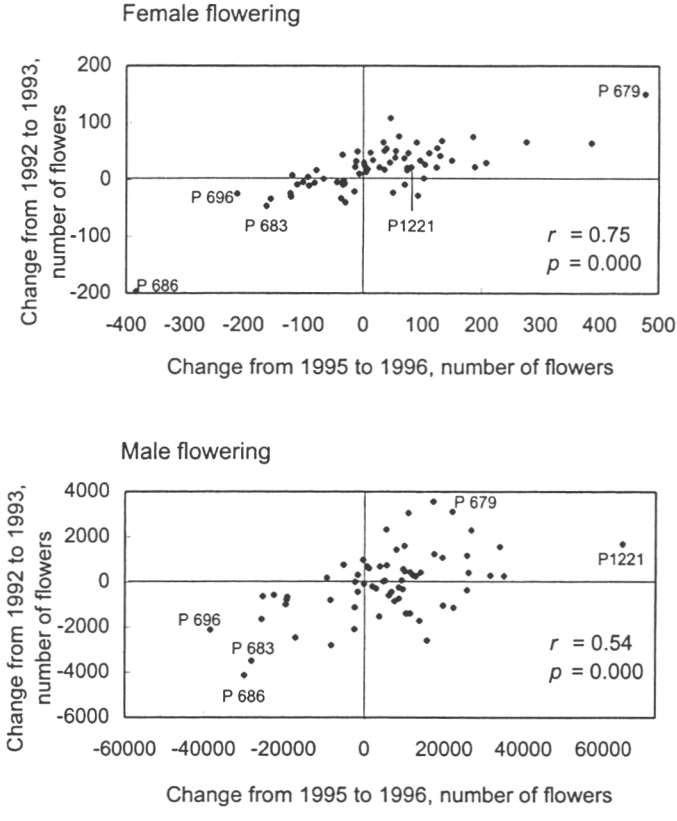

Fig. 4. Stability in the change of flowering abundance between two different pairs of years in the Heinämäki seed orchard. Some extreme clones are labelled.

than those for the height (0.30) and diameter (0.32) of the grafts.

Only the six fairly good flowering years (1987, 1989, 1992, 1993, 1995 and 1996) were included in a more detailed examination of clonal differences. The correlation coefficients between female and male flowering of the same years were always positive and statistically significant, with the exception of 1993 (Table 4). Also the correlation coefficients from year to year were usually positive and significant, and in general slightly higher in male than in female flowering (Table 4). However, in some cases the correlations between years were complex. In female flowering the correlation coefficients of successive years (1992 and 1993; 1995 and 1996) were rather low, 0.30 and –0.17, respectively, and in the latter case negative and not significant. On the other hand, when the first years of these two pairs of years (1992 and 1995), and correspondingly the second years (1993 and 1996) were compared, the correlation coefficients were significant and high, 0.64 and 0.73, respectively. The changes in flowering abundance between two successive good flowering years showed a persistent pattern for the two pairs of years (Fig. 4).

When the clonal differences in flowering were studied on the basis of geographic origin using correlation analysis, no correlation was found between female flowering and latitude, while there was a significant negative correlation between male flowering and latitude in 1989, 1993 and 1995 (Table 5). Male flowering decreased with increasing latitude of origin. The correlation coefficients between the average height of the grafts and flowering were significant in the studied years, except for 1993 and 1996 in female flowering, and for 1996 in male flowering. The

correlation coefficients between flowering and the other variables describing the graft size (diameter at breast height and crown volume) were very similar to those with height (Table 5). Clones with larger grafts had more abundant flowering.

3.3 Factors Affecting Flowering Abundance

The average number of female flowers per graft was 40% higher on the agricultural land than on the forest land, and the average number of male flowers 8% higher. For female flowering the difference was statistically significant ($p < 0.05$) in 1987, 1989, 1992 and 1993 (when six years of abundant flowering were analysed), but for male flowering only in 1996. The average height of the grafts in 1995 was 10.7 m (±0.11) on the agricultural land and 9.5 m (±0.13) on the forest land. The correlation between the height and flowering of the grafts was statistically significant ($p < 0.005$) for both female and male flowering in every year, the average Spearman rank correlation coefficient of six years for female flowering being 0.36 and for male flowering 0.44.

Differences in flowering abundance between the clones were investigated using stepwise regression analysis. The variables analysed were geographical latitude (lat) and longitude (lon) of the plus tree, average height (h), breast height diameter (dbh), crown width (wid) and crown volume (vol) of the grafts of each clone. The results for both female and male flowering in six separate years are shown in Table 6. The regression model for female flowering in 1992 included three statistically significant independent variables (dbh, in addition to vol and wid, $R^2 = 0.61$), while in the other cases there were two or less (Table 6). In general, female flowering was best

Table 6. Statistically significant regression equations with one and two independent variables for female and male flowering at the clonal level in the Heinämäki seed orchard.

Year	Intercept	First term			Second term			
		Variable	Coefficient	p	Variable	Coefficient	p	R^2
Female								
1987	–44.13	wid	2.67	0.001				0.15
1989	–4.04	vol	2.27	0.000				0.51
1989	175.13	vol	5.00	0.000	wid	–7.32	0.007	0.56
1992	–4.54	vol	1.31	0.000				0.51
1992	118.68	vol	3.19	0.000	wid	–5.04	0.001	0.59
1993	No significant regression							
1995	4.74	vol	1.65	0.000				0.23
1995	201.76	vol	4.65	0.001	wid	–8.05	0.029	0.29
1996	No significant regression							
Male								
1987	–1230	dbh	258.4	0.000				0.32
1989	–5255	dbh	707.6	0.000				0.31
1992	–3819	dbh	346.0	0.000				0.40
1992	–2520	dbh	213.6	0.011	vol	27.4	0.042	0.44
1993	–1150	dbh	172.6	0.000				0.21
1993	–784	dbh	294.4	0.000	wid	–69.0	0.013	0.29
1995	–36000	h	483.6	0.000				0.26
1995	–43652	h	372.0	0.001	lon	39.6	0.028	0.31
1996	–10287	dbh	1347.3	0.001				0.15
1996	–22790	dbh	2406.9	0.000	vol	–263.8	0.015	0.23

Note: wid = crown width, vol = crown volume, dbh = breast height diameter, h = graft height, lon = longitude of the clone origin

explained by crown volume, and male flowering by breast height diameter. The geographical origin of the clone was included in the model only in the case of male flowering in 1995.

3.4 Genetic Diversity in Seed Orchard Crops

The status number of the seed orchard was 56 when the variation in the number of ramets per clone was considered. This was equivalent to 84% of the number of clones (census number) in the orchard. The variation in female flowering had a considerable influence on the status number of the seed crop when the ramet number was assumed to be the same for all the clones, and male flowering was assumed to follow female flowering. The average status number after adjusting the variation in female flowering was 31 (46% of the census number), the variation between different years ranging from 12 to 48 (Table 7). The status number after adjusting for the variation in male flowering in the same way

Table 7. Estimated absolute (N_s) and relative (N_r) status numbers of the seed crop of the Heinämäki seed orchard after adjusting for different variation sources in the genetic contribution of the clones and for pollen contamination.

Year	Equal ramet number			Weighted with ramet number				
	Flowering of			Flowering of both genders				
	female	male	both genders	Percentage of pollen contamination				
				0	25	50	75	100
Absolute status number, N_s								
1984	22	28	31	32	40	50	61	75
1985	14	16	24	19	25	33	43	52
1986	12	33	23	17	21	25	31	37
1987	37	42	44	36	47	61	83	116
1989	41	43	48	37	48	63	87	121
1990	37	40	46	37	48	64	87	121
1991	24	31	37	31	39	49	62	76
1992	39	41	44	33	42	56	78	113
1993	48	49	55	42	55	75	105	153
1995	32	33	38	29	39	51	70	98
1996	31	44	44	36	44	56	73	95
Average	31	36	39	32	41	53	71	96
sd	11	9	9	7	10	14	21	34
Relative status number, N_r (%)								
1984	33	43	46	49	61	75	93	113
1985	21	24	36	29	38	51	65	79
1986	18	50	35	26	32	38	46	56
1987	56	63	64	55	70	92	125	176
1989	62	65	72	56	73	96	131	183
1990	56	60	70	56	73	97	132	183
1991	37	48	56	47	59	75	94	115
1992	59	62	66	50	64	85	118	171
1993	73	74	83	64	84	113	159	232
1995	49	50	57	44	58	77	106	148
1996	47	67	66	55	67	85	110	144
Average	46	55	59	48	62	81	107	145

as for female flowering was 36 (55%), ranging from 16 to 49. It was always higher than the status number adjusted for female flowering. When female and male flowering were considered together, the average status number was 39 (59%). When the status number of the seed crop was adjusted for all these three sources of variation, i.e. ramet number, female flowering and male flowering, it decreased to 32 (48%) on the average (Table 7).

The estimated effect of pollen contamination on the status number of the seed crop was large (Table 7). Even moderate pollen contamination (25%) increased the average status number from 32 to 41. The status number increased with increasing pollen contamination. With total background pollination it was 96, which is 45% higher than the census number of the seed orchard (Table 7).

The status number for the seed crop adjusted for variation in female flowering increased with increasing number of female flowers ($r = 0.65$, $p = 0.032$). For male flowering the corresponding dependence was weaker ($r = 0.29$, $p = 0.394$).

4 Discussion

In Norway spruce the between-year variation in flowering abundance and cone crop is large (Blomqvist 1876, Heikinheimo 1932, 1948, Tirén 1935, Koski and Tallqvist 1978). Many attempts have been made to explain this variation (e.g. Lindgren et al. 1977, Pukkala 1987). In our study the variation in female flowering could be explained reasonably well using Pukkala's (1987) model for predicting the seed crop index for southern Finland on the basis of the temperature data of the two previous summers. The greatest incompatibility between the prediction and the number of flowers in the seed orchard was observed in the two cases where there was good flowering in two successive years (Fig. 3). The model predicted a decreasing seed crop index for the latter years, but in these cases the flowering in the seed orchard actually increased. In the natural stands the abundance of flowering better followed the predicted value. The reason for the different behaviour of the seed orchard grafts was either their northern origin or the special conditions prevailing in the seed orchard.

The large, statistically significant variation between the clones in flowering abundance (Table 3) is in accordance with earlier results from Norway spruce in both natural stands and seed orchards (Sarvas 1968, Eriksson et al. 1973, Koski and Tallqvist 1978, Skrøppa and Tutturen 1985, Kjær 1996), as well as with other conifer species (Varnell et al. 1967, Jonsson et al. 1976, Bhumibhamon 1978, Koski and Tallqvist 1978, Schoen et al. 1986, Matziris 1993).

The broad-sense heritability estimates (Table 3) for female flowering were about the same or lower than those reported for the cone crop of other conifers (Varnell et al. 1967, Matziris 1993, Savolainen et al. 1993). Unfortunately, results for male flowering are scarce. The only comparable result concerns the heritability of pollen production in Scots pine (Savolainen et al. 1993), which was about the same as that for male flowering in our study. It is noteworthy that in our study with Norway spruce, as well as in the study of Savolainen et al. (1993) with Scots pine, the flowering characteristics had higher broad-sense heritabilities than height growth. The considerable amount of genetic variation in flowering characteristics is in contrast with the hypothesis of low variation in fitness-related characteristics (Falconer and Mackay 1996). This is not, however, the first time that this has been observed. Large genetic variation has been reported in other studies on flowering (see references above), as well as in other fitness-related characters (Harju et al. 1996). This apparent contradiction is discussed in other studies (Harju et al. 1996, Kjær 1996, Ruotsalainen 1998).

The broad-sense heritability estimates obtained in this study can be considered to be overestimates, because the seed orchard was not established using a random design. In the clonal-row design used here the ramets of a single clone were usually growing in two to four rows in different parts of the seed orchard. The effect of non-random distribution of the ramets on the heritability was examined by re-analysing the data after removing 12 clones with the most concentrated distribution. In most cases this data screening had no marked effect on the heritability estimates, but in the years with the highest

heritabilities the estimates decreased somewhat in both female and male flowering. Therefore the broad-sense heritability estimates can be regarded as rather reliable.

The finding that the correlation in female flowering between two successive good flowering years was poor (Table 4) is in accordance with the results for Norway spruce (Kjær 1996), white spruce (*Picea glauca* (Moench) Voss) (Schoen et al. 1986) and black pine (*Pinus nigra* Arnold) (Matziris 1993). In male flowering the changes in flowering abundance between successive years were not as great as those in female flowering, as also shown by Kjær (1996). The correlations for two pairs of successive good flowering years showed that there exist genotypes that have a different response to climatic factors: some clones flowered well in the first year, and other clones in second year. This tendency was especially clear in female flowering.

Our result that the same clones tend to have a large number of both female and male flowers (Table 4) is in accordance with earlier results for Norway spruce (Skrøppa and Tutturen 1985, Kjær 1996, Kjær and Wellendorf 1997), black spruce (*Picea mariana* (Mill) B.S.P.) (O'Reilly et al. 1982, Caron and Powell 1989) and white spruce (Schoen et al. 1986). Kang and Lindgren (1998) did not find any statistically significant correlation between female and male flowering in three pine species (*Pinus densiflora* Sieb. & Zucc., *P. thunbergii* Parl. and *P. koraiensis* Sieb. & Zucc.), but Nikkanen and Velling (1987) reported low positive correlation between female and male flowering in Scots pine. It should be kept in mind, however, that our results, as well as most of the other results cited above (with the exception of Kjær 1996), are based on phenotypic or clonal (genotype) means. In Scots pine the phenotypic and environmental correlations between female and male flowering are usually positive, but genetic correlation negative (Savolainen et al. 1993). However, the correlation between genotypic means gives a rather good approximation of the real genetic correlations if the genotypes are represented by a sufficient number of randomised ramets. Kjær (1996) also obtained from moderate to high genetic correlations between female and male flowering in a Norway spruce seed orchard. Thus there seem to be some differences in the mode of sexual allocation between spruces and pines, spruces having a more equal contribution to female and male flowering. One explanation for the differing correlations between female and male flowering could be that the correlation tends to be positive at a young age, but turns more negative with increasing sexual maturity (Savolainen et al. 1993). However, in our material there were no signs that the clones were specialising into different sexes with increasing age.

The differing land-use history of the central and outer parts of the seed orchard (agricultural vs. forest land) was reflected in many of the characteristics measured on the grafts. The grafts growing in the more fertile soil on the agricultural land (Table 1) were taller. The flowering abundance correlated in most cases with the size of the graft, and therefore the flowering abundance was affected by both environmental and clonal factors. The clonal variation in flowering was usually explained better by size characteristics of the grafts other than height; female flowering by crown volume and male flowering by breast height diameter (Table 5). The result that tall grafts with a wide crown had more flowers than small ones has also been obtained for grafts in a Scots pine clone bank (Nikkanen and Velling 1987).

Differences in the origin of the clones did not explain the variation in flowering (Table 5). This was contrary to expectations (Eriksson et al. 1973, Skrøppa and Tutturen 1985). In our study the origin of the clones may not have covered a sufficiently large area to show any clear differences in response to climatic adaptation. In the above studies the material covered large areas, consisting of provenances from Central Europe to Scandinavia.

When the factors affecting the clonal variation in flowering were examined by regression analysis, the overall result was that there was great year-to-year variation in the coefficient of determination (Table 6). When two pairs of successive good flowering years were studied, the coefficients of determination were smaller in the latter years. However, the heritability estimates for both female and male flowering were, on the average, slightly larger in the latter years of these pairs of years (Table 3). This could be due to

clonal variation unrelated to the size of the ramets or the origin of the clone. This, again, indicates differing genetic responses to factors regulating flower induction.

Our results demonstrate that the genetic diversity of the seed crop cannot be estimated only on the basis of the census number of the seed orchard, but that variation in the ramet number and flowering abundance as well as pollen contamination must also be considered (Table 7). When the variation in the number of ramets per clone was included, the status number of the seed orchard decreased to 84% of the census number. This decrease in the genetic diversity of a seed orchard is mainly caused by technical difficulties caused by lack of material, mortality etc., which prevent equal numbers of ramets being obtained for each clone. The decrease in status number caused by the variation in ramet number was smaller than that in Norway spruce seed orchards in Finland on the average (Kang et al. 2000), and within the range of variation observed in seed orchards of several other species (Kjær et al. 1995, Kang et al. 2000).

The variation in the abundance of female flowering decreased the status number more than the variation in male flowering (Table 7). The variation among years was also greater after adjusting female flowering than after adjusting male flowering. In Norway spruce, Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and noble fir (*Abies procera* Rehder) the relative status number of seed orchard crops after adjusting for the variation in female flowering varies considerably, but has usually been below 50% (Kjær et al. 1995, Kjær and Wellendorf 1998) which is in accordance with our results. In pines the relative status number has usually been higher than that for Norway spruce in our study (Kang and Lindgren 1998, Kjær and Barner 1998). The only available results concerning the effect of male flowering on genetic diversity indicated a lower decrease in status number in mature seed orchards of two pine species (*Pinus densiflora* and *P. thunbergii*) than in our study (Kang and Lindgren 1998). Our observation of the greater influence of variation in female than in male flowering was not unambiguously supported by the results for these pine seed orchards. Whether these results indicate systematic differences between pines and spruces is too early to say.

When both female and male contributions were adjusted together, the relative status number was slightly larger than that obtained after adjusting only for female or for male flowering (Table 7). In Norway spruce seed orchards in Denmark, the relative status numbers after adjusting for fertility variation in both genders was about the same as in our study (Kjær and Wellendorf 1998). According to Kjær et al. (1995), the effective clone numbers (equal to status number) of the seed crops of seed orchards of Norway spruce and noble fir always increase when both male and female flowering are adjusted. An increase in the status number of the seed crop after adjusting for both genders instead of only one, can be expected if there is sexual asymmetry between clones (Savolainen et al. 1993).

The status numbers, obtained after adjusting the fertility variation and weighted with the variation in the ramet number, were about half of the census number, with large annual variation. When pollen contamination was also taken into account, the status numbers clearly increased. The estimated level of pollen contamination in the studied seed orchard in three different years (1989, 1992 and 1993) is about 70% (Pakkanen et al. 2000). With this contamination level the status number of the seed crop after adjusting for all the existing variation would be the same as the census number of the orchard, and double the status number without pollen contamination. These results cannot be compared with those obtained in other studies because, as far as we know, the effect of pollen contamination on the genetic diversity of the seed orchard crop has not earlier been considered quantitatively. The results show that the level of pollen contamination has a great effect on the genetic diversity of the seed orchard crop. In our calculations pollen contamination was assumed to be derived from an infinite population of unrelated trees. If the fertilising pollen grains are related to each other or to the seed orchard clones, then the effect of pollen contamination will be smaller although still considerable (Lindgren and Mullin 1998).

In our study the differences between years with minimum and maximum status number were twofold when pollen contamination was not adjusted (Table 7). The genetic diversity of the

seed crop was the higher, the more abundant was the flowering. A similar result has been reported in other studies using either status number (Kjær and Wellendorf 1998) or other measures of genetic diversity (Ruotsalainen and Nikkanen 1989, Matziris 1993, Kjær 1996). In Scots pine the status number of the seed crop increases along with the seed crop with increasing age (Kjær and Wellendorf 1998), but in Norway spruce the development seems to be more erratic. According to our results, at an older age even a rather low flowering abundancy gives a more balanced seed crop than at a younger age (cf. years 1990–91 with 1984–86).

The results presented here do not concern the real seed crops, but have been predicted on the basis of flowering. However, there are several stages from flowering to seed crop that can affect the clonal contribution and thus the diversity of the seed crop (Sarvas 1968, Sweet 1975, Schoen et al. 1986, Schoen and Cheliak 1987). In a Norway spruce seed orchard the actual seed crop gave almost the same status number as the prediction based on the variation in flowering (Kjær and Wellendorf 1997). In a Sitka spruce seed orchard the relative effective clone numbers based on the number of cones and seeds differed considerably (Kjær et al. 1995). However, as also suggested by Kjær and Wellendorf (1998), monitoring the flowering abundance is a feasible means of obtaining a picture of the genetic diversity of seed crop. Differences between species can influence the feasibility of the method, and more comprehensive studies should be carried out on Norway spruce. Especially, the effect of male flowering and pollen contamination on genetic diversity should be clarified.

This study has demonstrated the large annual and clonal variation in female and male flowering in a Norway spruce seed orchard. On the basis of the differences in flowering abundance, the genetic diversity and the genetic composition of the seed crop varied from year to year. The estimate for the status number after adjusting for the variation in both female and male flowering was on the average 59%, and after adjusting for the variation in ramet number and estimated pollen contamination the same as the census number. The status number proved to be a feasible measure for describing the genetic diversity of the seed orchard crop. However, in order to be able to relate the level of genetic diversity of a seed orchard crop to the situation after natural regeneration, similar studies should also be conducted in natural stands.

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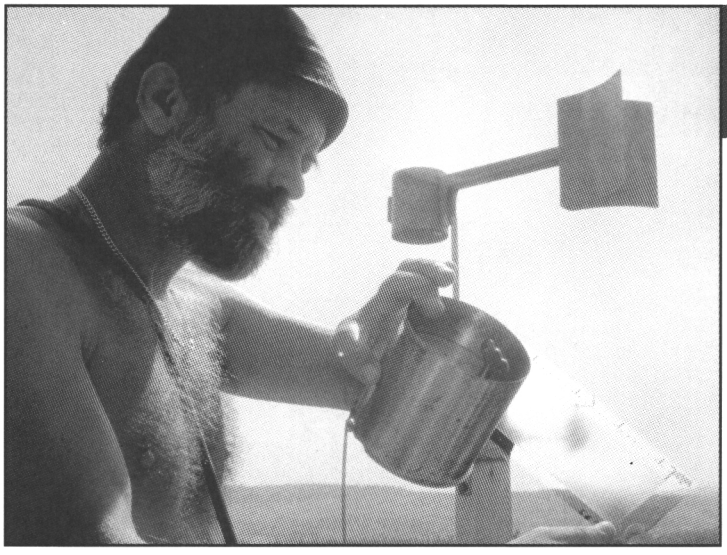

II

Reproductive Phenology in a Norway Spruce Seed Orchard

Teijo Nikkanen

Nikkanen, T. 2001. Reproductive phenology in a Norway spruce seed orchard. Silva Fennica 35(1): 39–53.

Reproductive phenology was studied in a Norway spruce seed orchard, located in southern Finland (62°13'N, 25°24'E), consisting of 67 clones from northern Finland (64°–67°N). Timing of flowering was determined on the basis of data recorded by a pollen catch meter during 1984–1995, and visual observations made on grafts in 1989, 1992, 1993 and 1995. The genetic and environmental factors affecting female and male phenology, and reproductive synchronisation were studied.

The between-year variation in the timing of flowering was more than three weeks. However, when it was defined on the basis of the effective temperature sum, the variation was smaller. No phenological reproductive isolation was found between the seed orchard and surrounding natural forests. The duration of the receptive period of the seed orchard varied from 5 to 8 days, and anthesis determined on the basis of airborne pollen from 5 to 10 days. The receptive period started about one day earlier than anthesis, except in one abnormally warm flowering period when female and male flowering started simultaneously. In general, the flowering periods of the different clones overlapped. The clonal differences in the phenology of receptivity were in most cases statistically significant, but in pollen shedding they were not. The broad-sense heritability estimates were higher for female than for male phenology. Environmental factors, conversely, had a stronger effect on male phenology. A wide graft spacing and a graft position that favoured solar radiation on the lower parts of the crown promoted early pollen shedding and, subsequently, better reproductive synchronisation between female and male flowering.

Keywords *Picea abies*, flowering, receptive period, pollination, reproductive synchronisation
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1 Introduction

Reproductive synchronisation, together with a balanced production of female and male strobili, random mating, minimal selfing and isolation from non-orchard pollen sources, are the basic biological assumptions when determining the genetic efficiency of wind pollinated seed orchards (Blush et al. 1993). However, many of these requirements are difficult to fulfil. In order to determine the degree of flowering synchrony of seed orchard clones, the reproductive phenology of a seed orchard has to be characterised. Data on the timing and duration of female receptivity and pollen release are needed for this purpose. Several different techniques have been developed for collecting and presenting data on reproductive phenology, the method applied depending on the species, conditions and accuracy requirements (Jonsson et al. 1976, Wheeler 1983, El-Kassaby et al. 1984, Griffin 1984, Erickson and Adams 1989, El-Kassaby and Reynolds 1990). In addition to knowing the variation in flowering phenology in the seed orchard, it is also important to be aware of the timing of pollen shedding outside the seed orchard compared to pollen shedding and female receptivity inside the orchard.

Numerous studies have shown that non-synchronous flowering is a serious problem in seed orchards of many coniferous species in the temperate region: Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (El-Kassaby et al. 1984, 1988, El-Kassaby and Askew 1991), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (El-Kassaby and Reynolds 1990), radiata pine (*Pinus radiata* D. Don) (Griffin 1984), loblolly pine (*Pinus taeda* L.) (Askew 1988, Askew and Blush 1990), and black pine (*Pinus nigra* Arnold) (Matziris 1994). In a colder climate, more simultaneous flowering has been reported in seed orchards of Scots pine (*Pinus sylvestris* L.) (Jonsson et al. 1976, Pulkkinen 1994, Burczyk and Chalupka 1997) and black spruce (*Picea mariana* (Mill) B.S.P.) (O'Reilly et al. 1982), but no information is available about Norway spruce (*Picea abies* (L.) Karst.) seed orchards. The only studies on the reproductive phenology of Norway spruce are the work carried out on flowering and the seed crop in natural stands by Sarvas (1968), the flowering study in a four-year-old clone trial by Eriksson et al. (1973), and the study on the climatic adaptation of Norway spruce in Finland by Luomajoki (1993).

The seed orchards in Finland have been established using clones originating from geographically and climatically limited areas (Sarvas 1970, Koski 1980, Nikkanen et al. 1999). This was done in order to ensure the adaptability of the seed orchard material to its utilisation area, which has usually been planned to be the same as that of the clone origins. Simultaneous flowering of the seed orchard was also aimed at by limiting the clone origin. Another measure directed at the phenology of the seed orchards was to locate the seed orchards of northern origin (like the studied one) in the southern parts of the country. In addition to enhanced flowering and better seed ripening, this was done in order to achieve phenological isolation between the seed orchard clones and surrounding forests (Sarvas 1970). The hypothesis was that the temperature sum required for the onset of flowering would be smaller in trees adapted to northern conditions than in those adapted to more southern conditions (Sarvas 1962, 1968, 1970). No phenological isolation has been found in Scots pine (Pakkanen and Pulkkinen 1991, Pulkkinen 1994), and no results from this or from any other measures directed at reproductive phenology in seed orchards have been reported for Norway spruce.

The aim of this study was to determine the phenological variation in female and male flowering in a Norway spruce seed orchard, and to describe the timing of the shedding of pollen. An additional aim was to determine the extent to which genetic and environmental factors affect flowering phenology, and to discuss the possible consequences of variation in reproductive phenology for the seed crop produced in the seed orchard.

2 Material and Methods

2.1 The Seed Orchard

The variation in flowering phenology was studied in Norway spruce seed orchard no. 170, Heinämäki, established in 1968 at Korpilahti,

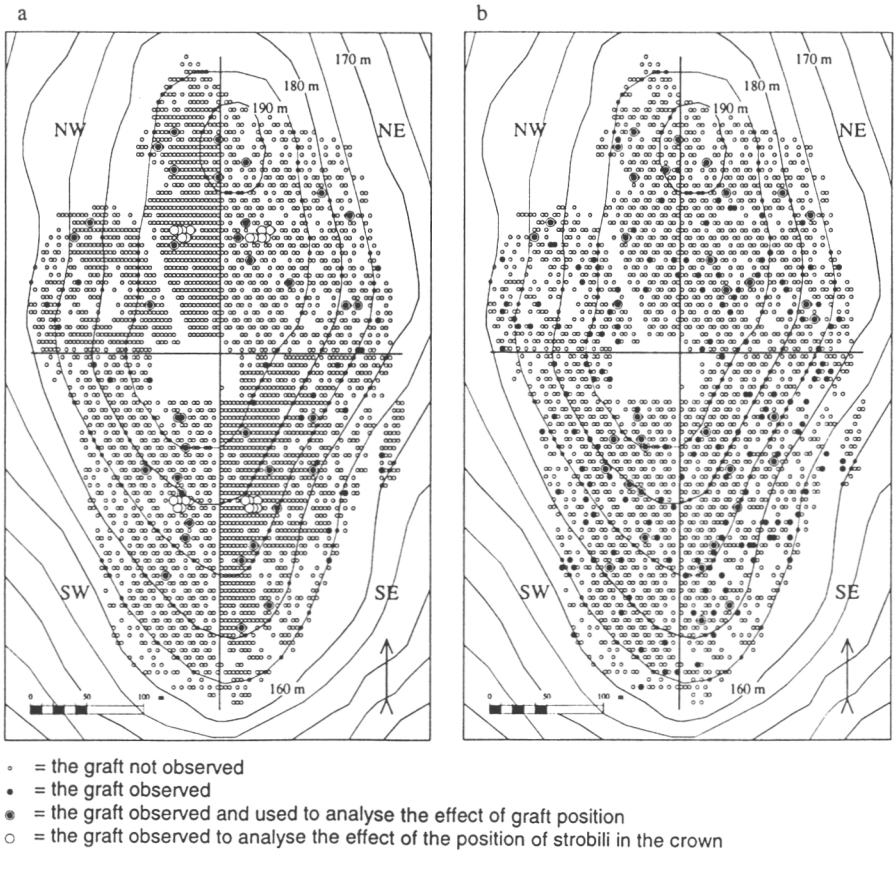

Fig. 1. The Heinämäki seed orchard before (a) and after (b) thinning in 1994. Grafts on which the phenological stage of the female and male flowers were observed are marked.

southern Finland (62°13'N, 25°24'E). The seed orchard consists of 67 clones originating from latitudes 64°–67°N in northern Finland (for more detailed information see Nikkanen and Ruotsalainen 2000).

The seed orchard is 13.2 ha in area, 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 spacing of the grafts was 3.5 × 6.5 m, the ramets of the same clone being located 6.5 m from each other. In 1987 half of the orchard was thinned systematically by removing every third graft, and in 1994 the other half of the orchard thinned in the same way. The different sections of the seed orchard before the thinning in 1994 were: north-western unthinned section (NW), north-eastern thinned section (NE), south-eastern unthinned section (SE), and south-western thinned section (SW) (Fig. 1).

The position of the grafts 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 studied area (Lähde et. al. 1992).

The spacing of the grafts was calculated by counting the number of grafts within a radius of 12.62 m (i.e. 500 m^2) around each graft. In addition, a slope index for the position of each graft was calculated for a circle of the same size. The slope index was calculated from the direction and gradient of the slope using formula (1).

$$I = 1 + \frac{\alpha - 5}{15} \times \frac{s - 90}{90} \quad (1)$$

where α is the slope angle (if $\alpha < 5°$ then $\alpha = 5°$ and if $\alpha > 20°$ then $\alpha = 20°$), s is the slope direction (deviation from north, degrees). The slope index has values ranging from 0 (north with 20°) to 2 (south with 20°). The spacing and the slope index were used as environmental factors to explain the differences in flowering phenology.

The measurements and results concerning the abundance of flowering and the growth characteristics of the grafts, e.g. height, diameter, and crown width, are given in Nikkanen and Ruotsalainen (2000).

The weather data for the study period were obtained from the Jyväskylä weather station (located 25 km north-east from the seed orchard) of the Finnish Meteorological Institute, where the temperature conditions were very close to those in the seed orchard. The weather data consisted of daily mean temperatures (including effective temperature sum, d.d., > +5°C) from 1984 to 1995, as well as cloudiness and precipitation during the flowering period.

2.2 Observations of Flowering Phenology

The timing and amount of airborne pollen were studied by means of a recording pollen catch meter (Sarvas 1962). The pollen catch was measured in the seed orchard from 1984 to 1995, and on a hill, where the environmental conditions were similar to those in the orchard, located about 1 km to the south-east from it from 1987 to 1995. The results obtained with the pollen catch meter provide information about the actual timing of flowering, the mean flowering day being the day when 50% of the total pollen catch was recorded. The duration of anthesis was defined by excluding pollen catches beyond the limits of –2 and +1.2 standard deviations, i.e. including in primary anthesis the period from 2.3 to 88.5% of the total pollen catch of the season (Luomajoki 1993). This was done in order to eliminate secondary pollen.

The phenological stage of the female and male flowers was observed on seed orchard grafts in 1989, 1992, 1993 and 1995. In 1989 the observations were made on 7 randomly chosen clones, in 1992 and 1993 on 21 randomly chosen clones with sufficient flowering abundancy, and in 1995 on 65 out of the 67 seed orchard clones. The observations on the phenological stage of the flowers were made on 3 grafts per clone. The sample grafts were the same in 1992 and 1993, and also in 1995 except for the few cases where they had been removed in thinning, had died or were not flowering. In those cases they were replaced with the nearest grafts of the same clone. This experimental design made it possible to study the effect of graft position in the seed orchard together with clonal variation. The effect of graft position was further studied by analysing randomly chosen grafts from four different sections of the seed orchard (Fig. 1), nine grafts per section in 1992, 1993 and 1995. In 1992 the effect of the position of strobili in the crown (height above ground level and exposure of the branch) was also studied on 24 separate sample grafts from 18 different clones (Fig. 1).

When studying the clonal and environmental variation in flowering phenology, the stage of development of the female strobili was determined by observing the top of the graft with binoculars, and the stage of the male strobili by observing pollen shedding from the sample branch on the southern side of the graft at a height of two to three meters. Phenological observations were made daily, or every second day depending on the weather and the stage of phenology. During warm, dry weather the observations were made every day whenever possible, and during cold, wet weather every second day. The observation round was planned to take no longer than two or three hours in order to ensure that the phenological stage would not have changed during the round. In 1995 when all the clones were included in the study, the observation round was divided into three separate rounds, each including one ramet per clone. The effect of the position of the strobili was investigated on 10 branches per graft by observing one branch at differing crown exposures in every flowering whorl.

The phenological stages of the female strobili were classified as follows: (0) strobili not yet receptive, i.e. completely protected by bud scales,

(1) strobili partly receptive, i.e. partly covered with bud scales and partly open, (2) strobili fully receptive, i.e. cone scales at a right angle and most of the ovules receptive, (3) strobili started to close, i.e. cone scales had started to bend upwards, and (4) strobili closed, i.e. all the cone scales bent. The stages for male strobili were: (0) pollen not yet shed, (1) small amount of pollen ready to be shed, (2) considerable amount of pollen being shed, and (3) almost all pollen shed.

2.3 Data Analysis

In 1992 when the effect of flower position in the crown was investigated, the phenological stages were used in the calculations as such, while in all the other cases the dates when a certain phenological stage had been reached were used in the calculations. The dates of stage 2 in both female and male phenology represented the timing of female receptivity and of pollen shedding. When observations were not made every day, the date of the phenological stage was interpolated.

When the dates of the phenological stages were used in the analysis, a non-parametric Kruskall-Wallis test was used to determine the statistical differences between the clones, and the Spearman rank correlation procedure to calculate the strength of the linear association between different variables. Because the used day scale was so coarse that the observations fell in only a few classes, a non-parametric test and rank correlation procedures were used. When the phenological stages were used instead, the normal score transformation was performed before the analysis of variance and the Tukey post-hoc test using the GLM General Factorial procedure. All the analyses were performed by SPSS® Base 8.0 statistical software (SPSS Inc. 1998).

Broad-sense heritabilities (h_B^2) (= clonal repeatability) were estimated on the basis of a single graft using the formula of Sokal and Rohlf (1995, p. 214) as described in Nikkanen and Ruotsalainen (2000).

3 Results

3.1 Variation in the Timing of Flowering

The between-year variation in the timing of pollen shedding was large (Fig. 2). When the years with poor anthesis were excluded and seven years (1985, 1986, 1987, 1989, 1992, 1993 and 1995) out of the twelve were examined, the mean flowering date varied from May 15 to June 6, the average being May 28. The effective temperature sum of these dates varied from 122 d.d. to 159 d.d., the average being 141 d.d. (Table 1). The timing of anthesis measured outside the seed orchard did not differ from that in the orchard. The duration of primary anthesis varied from 5

Table 1. Duration of primary anthesis, the weather conditions and the effective temperature sums during primary anthesis in the Heinämäki seed orchard in different years.

Year	Duration of anthesis days	Number of rainy days	Number of sunny hours [1]	Mean [2] temperature °C	Timing of anthesis Start	Median d.d.	End
1985	8	3	7.7	8.3	100	124	130
1986	7	6	5.3	9.6	130	159	162
1987	8	6	3.5	12.5	89	134	149
1989	10	3	12.1	12.0	79	122	148
1992	6	0	14.3	17.0	101	148	173
1993	6	2	9.4	13.8	108	143	163
1995	5	1	13.2	20.1	93	152	169
Average	7	3	9.4	13.3	100	141	156

[1] per day
[2] during the anthesis

to 10 days, the average being 7 days.

During the seven years examined, there were large differences in the onset of spring and in the weather during flowering (Table 1, Fig. 2).

Both female and male phenology was observed visually on the seed orchard grafts in 1989, 1992, 1993 and 1995. The time difference in the start of the receptive period of the female flowers between the earliest and the latest graft varied from 2 (1995) to 4 (1993) days, and in the start of pollen shedding from 3 (1995) to 6 (1993) days. The average duration of the receptive period of the grafts was 4.0, 3.7, 4.0 and 2.6 days in 1989, 1992, 1993 and 1995, respectively. The receptive period of the whole seed orchard varied from 5 (1995) to 8 (1993) days. On the average, the receptive period of an individual graft started from 4 (1989) to 0 (1995) days earlier than pollen shedding on the same graft.

3.2 Clonal Differences in Flowering Phenology

The phenological observations made in 1992, 1993 and 1995 were used in the statistical analyses. The clonal differences in the start and duration of the receptive period were statistically significant ($p < 0.05$) in all cases, apart from the start of receptivity in 1992 (Table 2). The average broad-sense heritabilities for the start and duration of the receptive period were 0.28 and 0.36, respectively, and for the start of pollen shedding 0.17 (Table 2).

The Spearman rank correlation coefficients of the clones between the years in the start and the duration of the receptive period were positive and in most cases statistically significant. The correlation coefficients between the start and duration of the receptive period were always negative, and in 1992 and 1995 statistically significant (Table 3a), i.e. the early clones had a longer receptive period than the late ones. The ranking of the clones between the years in the start of pollen shedding was statistically significant in all cases (Table 3b). The ranking of the clones between female and male phenology was statistically significant only in 1992 (Table 3c).

The differences between the clones in the start of the receptive period were 1, 3 and 1 day and in

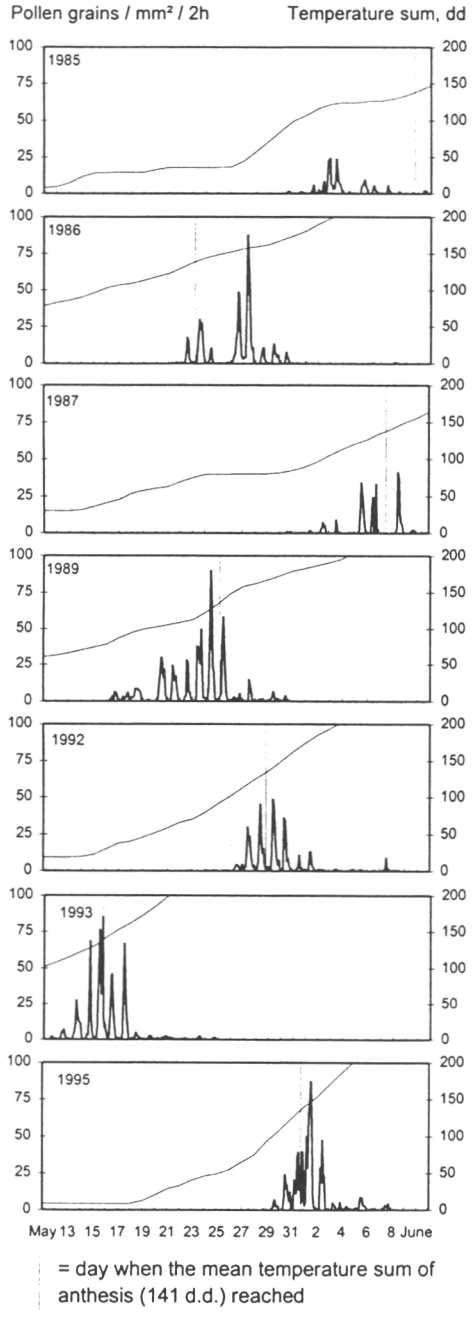

Fig. 2. The number of pollen grains captured in the Heinämäki seed orchard during flowering seasons of moderate or abundant anthesis, and accumulation of the effective temperature sum (> 5°C) based on temperature measured at Jyväskylä Weather Station.

Table 2. The significance of clonal differences in the Kruskall-Wallis test, and the broad-sense heritability for the start of receptivity and pollen shedding and for the duration of receptivity in the Heinämäki seed orchard.

Year	Significance of clonal differences			Broad sense heritability		
	♀ Start	♀ Duration	♂ Start	♀ Start	♀ Duration	♂ Start
1992 [1]	0.090	0.014	0.062	0.22	0.42	0.21
1993 [1]	0.019	0.022	0.109	0.41	0.41	0.24
1995 [2]	0.030	0.010	0.332	0.21	0.26	0.05

[1] data from 21 clones
[2] data from 58 (♀) and 60 (♂) clones

Table 3. The Spearman rank correlation coefficients of 21 clones (significance in parentheses) between years a) in the start and duration of the receptive period, b) in the start of pollen shedding, and within different years c) in the start of the receptive period and pollen shedding in the Heinämäki seed orchard.

a

Female	Start 1992	Start 1993	Start 1995	Duration 1992	Duration 1993
Start, 1993	0.58 (0.006)				
Start, 1995	0.44 (0.050)	0.35 (0.130)			
Duration, 1992	–0.53 (0.014)	–0.26 (0.254)	–0.24 (0.300)		
Duration, 1993	–0.31 (0.173)	–0.32 (0.155)	–0.05 (0.829)	0.66 (0.001)	
Duration, 1995	–0.36 (0.121)	–0.22 (0.354)	–0.66 (0.002)	0.71 (0.000)	0.54 (0.014)

b

Male	Start 1992	Start 1993
Start, 1993	0.45 (0.040)	
Start, 1995	0.49 (0.028)	0.45 (0.046)

c

	Start 1992	Start 1993	Start 1995
Female × male	0.53 (0.013)	0.26 (0.254)	0.38 (0.101)

Table 4. The average phenological stages of female and male strobili in different crown exposures and heights in 1992, and significance for differences in ANOVA after normal score transformation. The average phenological stages marked with different letters differ significantly from each other, $p < 0.05$ in the Tukey post-hoc test.

Exposure and height in the crown			Gender and date of observations	
			♀ May 26 or 27	♂ May 28
			Phenological stage of flowers	
	N		a 1.33	a 1.71
W + E		> 4 m	a 1.17 + 1.40 a	a 1.79 + 1.77 a
	S		1.59 a	1.80 a
p for differences between exposures			0.840 [1]	0.900 [2]
	N			a 1.30
W + E		≤ 4 m		a, b 1.50 + 1.86 b
	S			1.66 b
p for differences between exposures				0.010 [3]

[1] data from 51 observations
[2] data from 136 observations
[3] data from 90 observations

the start of pollen shedding 3, 5 and 2 days in 1992, 1993 and 1995, respectively. The average duration of the receptive period of the clones was 4.2 (varying from 3 to 5), 5.0 (3–7) and 3.2 (2–5) days in 1992, 1993 and 1995, respectively.

No correlation was found between female or male phenology and the geographic origin of the clones. Neither was there any correlation between the phenology and the number of flowers, except in 1995 when the receptive period started the earlier ($r = -0.30$, $p = 0.022$) the more abundant was the flowering. When the average size of the grafts and the phenology was examined, statistically significant correlation was found between the crown volume and the start of receptivity in 1995 ($r = 0.40$, $p = 0.002$), and the start of pollen shedding in 1995 ($r = 0.30$, $p = 0.021$) and in 1993 ($r = 0.52$, $p = 0.016$), i.e. flowering started later in the clones with a large crown.

3.3 Environmental Effects on Flowering Phenology

In 1992 the receptive period in the whole graft usually started within one day, but there were 1 to 3 days differences in the start of pollen shedding. In the strobili situated in the upper part of the crown (> 4 m) pollen started to shed earlier than in the lower part. In the lower part the exposure in the crown also affected pollen shedding (Table 4).

The differences in flowering phenology between the randomly chosen grafts growing in different sections of the seed orchard (see Fig. 1) were more significant in male than in female phenology (Table 5). In the northern sections of the orchard pollen shedding took place later than in the southern sections.

Environmental factors had a stronger effect on male than on female phenology also when the larger data set for 1995 was examined. The slope index (the direction and gradient of the slope)

Table 5. The average number of days, counting from May 1, when the receptive period and pollen shedding started in four different sections (9 grafts / section) of the Heinämäki seed orchard, and the statistical significance of the differences in the Kruskall-Wallis test.

Section of the seed orchard	Start of receptive period Year			Start of pollen shedding Year		
	1992	1993	1995	1992	1993	1995
NW Thinned in 1994	26.0	13.1	31.1	28.0	16.0	31.7
NE Thinned in 1987	26.0	13.7	31.2	27.8	15.1	31.8
SE Thinned in 1994	25.7	12.4	30.9	26.8	13.9	31.1
SW Thinned in 1987	25.8	13.2	31.0	26.8	14.0	31.1
p for differences between sections	0.264	0.081	0.476	0.001	0.021	0.003

Table 6. The Spearman rank correlation coefficients of the grafts (significance in parentheses) between the start of receptivity and pollen shedding, and some environmental factors.

Phenology in 1995	Altitude of grafts	Spacing of grafts	Slope index	Spacing / slope index
Start of [1] receptivity	0.25 (0.001)	0.20 (0.012)	–0.07 (0.363)	0.20 (0.010)
Start of [2] pollen shedding	0.40 (0.000)	0.26 (0.001)	–0.22 (0.004)	0.28 (0.000)

[1] data from 162 grafts
[2] data from 171 grafts

correlated significantly with the start of pollen shedding, but not with the start of female receptivity (Table 6). Pollen shedding started the earlier, the more southerly directed and the steeper was the slope, and the later the more northerly directed and the steeper it was. The spacing of the grafts correlated with both female and male phenology such that flowering started earlier when the spacing was wide. The correlations were stronger when the spacing was weighted by the slope index. In addition, the position of the graft was significantly correlated with both female and male phenology, i.e. the higher the position the later flowering.

3.4 Reproductive Synchronisation

Pollen shedding on the first grafts in the seed orchard usually started at about the same time as female receptivity. On the average, however, the female flowers developed earlier than the male flowers, because the proportion of receptive grafts or clones increased at a faster rate than the proportion of grafts or clones shedding pollen, except in 1995 (Fig. 3). All the clones in the seed orchard were simultaneously receptive on at least one day. On this day (May 26 in 1992, May 14 in 1993 and May 31 in 1995) 62, 81 and 88% of the clones, respectively, had started to shed pollen. The proportion of pollen captured by the pollen catch meter on that day was 3, 12 and 20%, and up to that day 4, 26 and 33% of the total pollen catch, respectively. In 1992 all the 21 clones were able to participate in the pollination of all the clones, but in 1993 four out of the 21 clones (P391, P1208, P1217 and P2306) had passed receptivity before the last three clones (P391, P496 and P2578) had started to shed pollen. In 1995 all 60 clones producing male strobili were able to pollinate all 58 clones bearing female strobili.

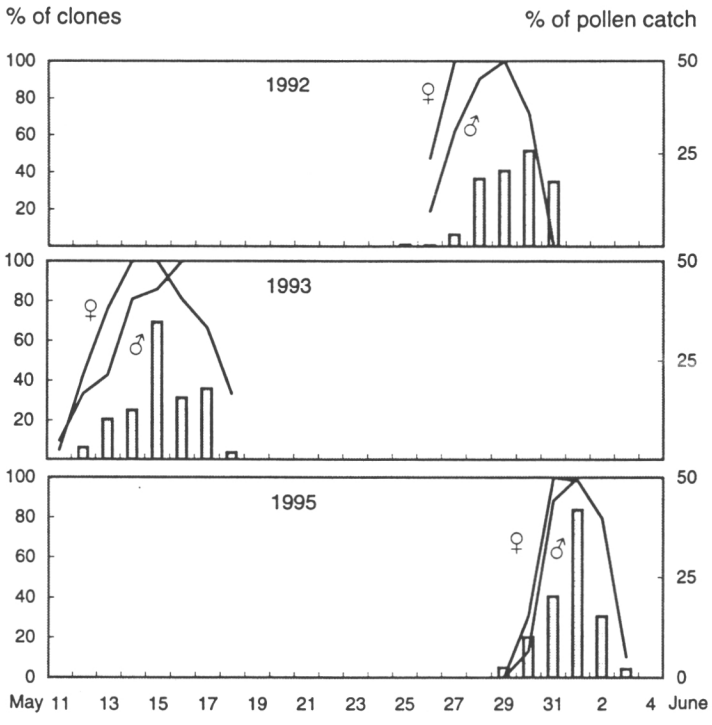

Fig. 3. The development of female receptivity and pollen shedding at the clonal level, and the proportions of pollen catches (bars) by pollen catch meter in 1992, 1993 and 1995.

4 Discussion

In southern Finland, Norway spruce flowers in the latter half of May or in the beginning of June. The midpoint date of anthesis, which represents the average date of flowering, varied in this study from May 15 to June 6, the average date being May 28 (Fig. 2). The average temperature sum that had accumulated by these days was 141 d.d.(Table 1), which was the same temperature sum as Sarvas (1968) obtained for a natural stand in southern Finland in two different years, and close to the figure (134 d.d.) Luomajoki (1993) obtained for a natural stand located near the seed orchard. The range of 37 d.d. between the lowest and highest temperature sum values for the midpoint dates of anthesis corresponds to 6–7 days calculated using the average daily temperatures during the flowering period. This is much smaller than the difference of 23 days observed in this study. The result that the temperature sum explained the timing of flowering better than the date is in accordance with the findings and conclusions of Sarvas (1968, 1972) and Koski (1991), but contrary to that of Luomajoki (1993).

Anthesis lasted from 5 to 10 days, which is several days less than Luomajoki (1993) obtained for natural stands. The difference is probably due to the more strict delimitation of primary anthesis in this study (2.3–88.5% of the total pollen catch). After the primary period a small amount of pollen, probably partly derived from the surface of the branches and the ground, was caught during a period of 5 to 7 days.

The duration of the receptive period in the seed orchard varied from 5 to 8 days in different years (Fig. 3). The duration of receptivity in the different clones varied from 2 to 7 days depending on the clone and the year. There are no data available from Norway spruce seed orchards

which could be compared with these results. Other results about the receptivity of Norway spruce are also scarce. On the basis of unpublished data collected by the late Professor Risto Sarvas, the receptive period of a stand of 30 trees at Punkaharju lasted for 16, 9 and 10 days in 1966, 1967 and 1968, respectively. In Sweden, Eriksson et al. (1973) found that the receptivity of Norway spruce clones in a young clone trial varied from 7 to 12 days (the whole period lasted 13 days) in 1971 during rather cold weather. Eriksson et al. (1973) discussed how to define receptivity with several examples and figures, and concluded that each model is still an oversimplification since often only a part of the ovules in a strobilus are receptive at one time.

The short and rather simultaneous flowering period observed in this Norway spruce seed orchard is different from that reported for many other conifer species. In Douglas fir seed orchards the duration of flowering period varies from 3 to 4 weeks (El-Kassaby et al. 1984, El-Kassaby and Askew 1991), and the duration of receptivity among families between 5 and 12 days (El-Kassaby and Askew 1991). Sitka spruce functions in the same way, i.e. the duration of the receptive period of the whole seed orchard is about 30 days (El-Kassaby and Reynolds 1990), while in individual clones it is much shorter and in individual strobili it lasts from 6 to 8 days (Owens and Blake 1984). Black spruce and white spruce are more similar to Norway spruce. In a black spruce seed orchard the receptive period of 12 clones lasted 16 days, the average length of the individual clone being 12 days including also partial receptivity (O'Reilly et al. 1982). In white spruce (*Picea glauca* (Moench) Voss) the receptive period for a single strobili is 10 days (Ho 1984). The reproductive phenology of Scots pine, the other common north European conifer, is somewhat different from that of Norway spruce. According to Chung (1981), the receptive period of a female strobilus is about one week (from 3 to 10 days), but the differences between clones are, in many cases, more than one week in a clone bank in southern Finland. In Sweden, Jonsson et al. (1976) found clonal differences to be considerable in a Scots pine seed orchard where the flowering period lasted from 2 to 3 weeks in four different years. Pulkkinen (1994) reported clearly shorter periods for two seasons in a Scots pine seed orchard of northern Finnish origin located in southern Finland. In other pine species the flowering periods are often longer than in Scots pine or in Norway spruce (Nilsson 1981, Griffin 1984, Askew and Blush 1990, Matziris 1994).

In this study female phenology was genetically more strongly determined than male phenology (Table 2). The clonal differences at the start of receptivity were, in most cases, statistically significant but not in pollen shedding, and the broad-sense heritability estimates were higher for female than for male phenology. This can also be looked in the opposite way: the environmental factors had a stronger effect on male than on female phenology. In studies on reproductive phenology, very little attention has been paid to environmental factors. The reason for this has been the relatively small variation between ramets compared to that between clones in many species and seed orchards (Jonsson et al. 1976, Wheeler 1983, Griffin 1984, El-Kassaby et al. 1984, Matziris 1994). For Douglas fir, Erickson and Adams (1989) estimated from Wheeler's (1983) data that the repeatability among ramets within clones for the timing of receptivity was extremely high (0.94). In radiata pine the clonal repeatability for the onset of receptivity was 0.42 and for the start of pollen shedding 0.33 over three years (Griffin 1984), and in black pine the corresponding repeatabilities were 0.69 and 0.23, respectively over two years (Matziris 1994). In the present study the average repeatabilities, i.e. broad-sense heritabilities, over three years were 0.28 and 0.17 for receptivity and pollen shedding, respectively.

Although the clonal differences in flowering phenology in the present study were rather small and affected by environmental factors especially in the case of male flowering, in most cases the ranking of the clones was similar from year to year (Table 3). The correlation coefficients between female and male phenology were positive, but statistically significant only in one year. No results from Norway spruce trees or clones showing the correlation of flowering phenology between years or between female and male phenology within the same year have earlier been presented, but the phenomenon is well known in other coniferous species. In Scots pine the corre-

lation in flowering phenology between clones from year to year is positive and significant (Jonsson et al. 1976, Chung 1981, Pulkkinen 1994, Burczyk and Chalupka 1997). It has also been shown for Douglas fir (El-Kassaby et al. 1984), radiata pine (Griffin 1984), loblolly pine (Askew 1988), and black pine (Matziris 1994), that the order of the onset of receptivity and pollen shedding among clones remains unchanged.

Differences in the origin of the clones did not explain the clonal differences in flowering phenology, as was also the case in flowering abundance (Nikkanen and Ruotsalainen 2000). In this study the origin of the clones may not have covered a sufficiently large area to show any clear differences in response to climatic adaptation. On the other hand, Eriksson et al. (1973) did not find any significant differences in the onset of the receptivity between origins even though their material covered large areas, i.e. clones ranging from Central Europe to Scandinavia.

In Norway spruce the female flowers are mainly situated at the top of the tree, but the male flowers also in the lower parts. There are obvious differences between the upper and lower part of the graft as regards solar radiation, air flow and humidity. At the top of the grafts the environmental conditions are about the same irrespective of the exposure of the crown and the section of the orchard, but in the lower parts the conditions differ. The findings concerning the differences in the onset of pollen shedding (Table 4) were in accordance with earlier results for Scots pine, i.e. earlier pollen shedding on the southern than on the northern side of the crown (Jonsson et al. 1976), and in the upper and middle parts exposed to the sun than in the lower parts of the crown (Chung 1978). The other finding that pollen shedding started earlier on the eastern than on the western side of the crown was probably due to the warming and drying effect of sunshine on the eastern side before the observations were made in the morning.

Variation between the different parts of the seed orchard was also found in the start of pollen shedding, but not in the start of the receptive period. On the average, pollen shedding started about 1 day earlier on the southern slope than in the northern parts of the orchard (Table 5). When the environmental factors were investigated in more detail in 1995, it was found that both the spacing of the grafts and the slope index (direction and gradient of a slope) affected male phenology especially (Table 6). These results indicate that the wider the grafts are located, the earlier will pollen shedding start, and also that the more southerly directed and steeper the slope is, the denser will it have to be to have the same environmental effect on the start of pollen shedding.

The result that flowering, especially pollen shedding, started later in the clones with a wide crown could be another expression of the need for solar radiation or high temperature. Because the clonal differences in the crown size are large (Nikkanen and Ruotsalainen 2000) and the ramets of the same clone were planted side by side in a north-south direction, the grafts of the wide-crown clones were often overshadowed by the grafts of the same clone, and the flowers on the shaded side of the graft by the graft itself. In addition to the genotype, crown size was also affected by the environment.

The synchronisation of female and male flowering varied from year to year (Fig. 3). In 1995, as a result of the very warm weather, the duration of flowering was short, and the time difference between the clones was small. In this year female and male flowering took place completely simultaneously, and the amount of pollen in the air was also high right from the very beginning of the receptive period. In other years flowering lasted longer, the clonal differences in the timing of flowering being larger and female receptivity developing earlier than pollen shedding. In 1993 some of the 21 clones had passed their receptivity before some of the clones had started to shed pollen. This means that, at least in some years, some of the clones do not participate in the pollination of all the clones in the seed orchard.

It was not possible, on the basis of the results from the pollen catch meters inside and outside the seed orchard, and on the visual observations made on flowering phenology in the seed orchard, to distinguish any time difference between the airborne pollen of non-orchard origin and that released from the seed orchard grafts. The results of the isozyme analysis of the seed from the same seed orchard, which showed that the pollen contamination rate was about 70% in 1989,

1992 and 1993 (Pakkanen et al. 2000), also indicate that there is no phenological isolation between the seed orchard and the surrounding natural forests, as had been assumed by Sarvas (1970). Neither has any isolation been achieved in the case of Scots pine. In the seed orchards of northern Finnish origin the rates of pollen contamination have been 33% (Harju and Muona 1989) and from 45 to 76% (Pakkanen and Pulkkinen 1991) in different orchards and in different years. In addition to a lack of phenological isolation, Pulkkinen (1994) has proposed that one of the reasons for the high pollen contamination would be metandry, i.e. the phenomenon in which the female flowers are receptive before the male flowers on the same trees shed pollen, which is characteristic for both pine and spruce (Sarvas 1968). According to Pulkkinen (1994), this is even overemphasised in Scots pine seed orchards of northern origin established to the south. Harju and Nikkanen (1996) have shown that, when pollination in Scots pine seed orchard is restricted to the pollination peak, pollen contamination is lower than during the period of less abundant pollen release at the beginning of female receptivity. This also indicates that delayed pollen shedding of the seed orchard grafts could be one reason for the high pollen contamination.

This study has demonstrated that wide spacing of grafts promotes early pollen shedding. The position of the grafts on the southern slope also has a similar effect and shortens the time difference between female receptivity and pollen shedding. Pakkanen et al. (2000) found that pollen contamination in the thinned parts of the seed orchard is in some cases lower than that in the unthinned parts. All this suggests that it is essential in Norway spruce seed orchards to keep the orchard open enough to ensure more solar radiation and better ventilation for the lower parts of the crown. Adequate thinning can be used to promote early pollen shedding and decrease pollen contamination through better reproductive synchronisation and, subsequently increase the genetic efficiency of the seed orchard.

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Variation in pollen viability among *Picea abies* genotypes – potential for unequal paternal success

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Abstract An in vitro germination method was used to study variation in pollen viability, that is pollen-tube growth rate together with germination percentage, among the *Picea abies* genotypes in a seed orchard. The method permits easy, rapid screening of large numbers of genotypes. Significant variation in pollen viability among the genotypes was evident, the differences among the pollen-lot means being 7–10–fold in different years. No correlation was found between the average pollen viability and the phenology, growth or growing-site characteristics of the pollen donors. However, there appeared to be pollen lots that either benefit from a higher germination temperature or else germinate faster at lower temperatures. The significant variation in pollen viability among the pollen donors indicates a potential for male gametophyte competition. This, together with the observed genotype-environment interactions in pollen performance, may contribute to the variable genetic composition of seed produced in the seed orchard.

Key words Norway spruce · Pollination · Germination of pollen · Pollen-tube growth · Functioning of seed orchard

Introduction

The fitness of male gametophytes depends both on paternal traits, which include the phenology of male flowering and the amount of pollen produced, and on pollen grain traits, such as the germination percentage, germination time, pollen-tube growth rate and selective fertilisation (Pfahler 1975). Competition among male gametophytes has been extensively studied and discussed in angiosperms (Mulcahy 1983 and references therein; Charlesworth 1988; Quesada et al. 1993). In *Hibiscus moscheutos*, for example, a faster average pollen-tube growth rate of the pollen donor has been shown to result in the siring of a larger number of seeds (Snow and Spira 1991, 1996). Also, in several gymnosperm species, e.g. *Pseudotsuga menziesii, Pinus radiata, Pinus taeda* and *Picea abies,* the application of pollen mixtures has resulted in unequal paternal success of the pollen donors. Pollen competition, including different rates of germination and tube growth, has been suggested as one of the reasons for this (Schoen and Cheliak 1987; Nakamura and Wheeler 1992; Skrøppa and Lindgren 1994). So far the only report of gymnosperms actually showing differences in the average pollen-tube growth rate among pollen donors is in *Pinus sylvestris* (Venäläinen et al. 1999), but there is no information about whether variation in this trait could also affect the genetic composition of the seed produced in seed orchards.

A seed orchard is a plantation of genetically superior trees, managed to produce frequent, abundant and easily harvested seed crops. Such an orchard is established by setting out clones or the seedling progeny of trees selected for desired characteristics (Zobel et al. 1958). In order to ensure the production of genetically diverse and physiologically high-quality seed crops, seed orchards have to fulfil certain requirements with respect to flowering and pollination in the orchard. The functioning of forest-tree seed orchards is often far from ideal. There are large differences in flowering abundance between clones and from year to year in several species (Sweet 1975; Jonsson et al. 1976; Bhumibhamon 1978), including *P. abies* (Lindgren et al. 1977; Skrøppa and Tutturen 1985). Also variation in the reproductive phenology has an effect on the genetic composition of seed produced in seed orchards (Chung 1981; Blush et al. 1993; Harju and Nikkanen 1996). Owing to the abundance of the species and effective pollen dispersal (Koski 1970; Lindgren et al. 1995), high pollen contamination has proved to be a serious problem in the functioning of seed orchards of *P. sylvestris* and *P. abies* (El-Kassaby et al. 1989; Harju and Muona 1989; Savolainen 1991; Pakkanen and

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Pulkkinen 1991; Paule et al. 1993; Pakkanen et al. 2000). In addition, the genetic composition of the seed produced in seed orchards may be affected by competition among pollen grains.

The aim of the present study was to determine whether there is variation in pollen viability, that is pollen-tube growth rate together with germination percentage, among the *P. abies* genotypes in a seed orchard and, if such variation is found, whether it is connected with other characteristics of the pollen donors or any exogenous factors. The germination percentage and the pollen-tube growth rate of different pollen lots were studied using an in vitro germination method that permits easy, rapid screening of large numbers of trees. In addition, the germination conditions were varied in order to study the behaviour of pollen lots under varying environmental conditions.

Material and methods

The seed orchard

The variation in pollen-tube growth was studied in Norway spruce (*P. abies*) seed orchard no. 170 (Heinämäki) located at Korpilahti, southern Finland (62°13'N, 25°24'E). It consists of 67 clones originating from latitudes 64°–67°N in northern Finland (Nikkanen et al. 1999). The seed orchard, 13.2 ha in size, was established in 1968 on a hill on abandoned agricultural land (Fig. 1).

A number of the properties of this seed orchard have been studied: the variation in flowering abundance (Nikkanen and Ruotsalainen, unpublished), the phenology of flowering (Nikkanen, unpublished), and pollen contamination (Pakkanen et al. 2000). The data from progeny tests of the clones are also available (Ruotsalainen and Nikkanen 1999).

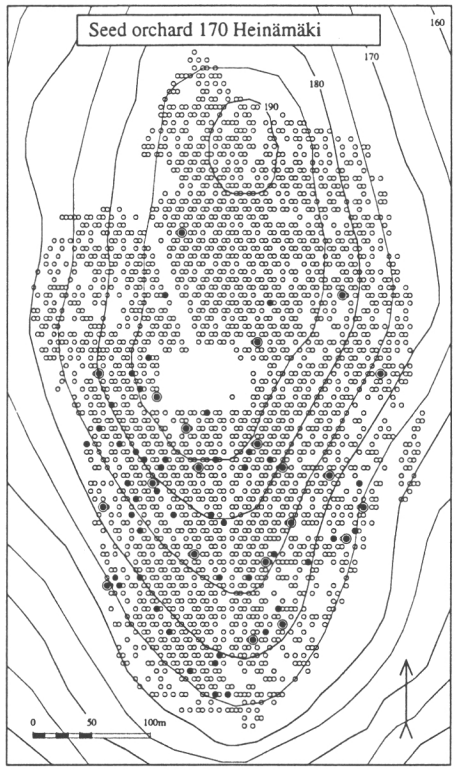

Fig. 1 A map of Norway spruce seed orchard no. 170 indicating the location of the pollen-donor genotypes (●, ◉). The 21 genotypes used in studying the effects of varying germination conditions are marked with ◉

Collection and storage of pollen

Pollen samples were collected from 66 of the 67 clones in the seed orchard in 1996 and 1998. A single graft from each clone was selected as pollen donor (Fig. 1). The same grafts were used in both years. In addition to the seed-orchard clones, five trees from surrounding areas were used as pollen donors, but these trees were not the same in 1996 and 1998.

Pollen was collected by isolating microsporangiate strobili with paper bags a few days before natural pollen shedding. Pollen collected in 1996 was stored in sealed glass bottles at –20°C. Samples from pollen collected in 1998 were taken directly from the isolation bags for in vitro germination, and the rest of the collection was stored as in 1996.

In vitro germination of pollen

Pollen lots were germinated in vitro in 24-well plates by suspending 10 mg of dry pollen in 1 ml of modified Brewbaker's and Kwack's (1963) medium, the suspensions being agitated on an orbital shaker (Infors AG, 180 rpm) as described by Häggman et al. (1997). The germination time, temperature and illumination during germination varied, but in all the experiments each pollen lot was germinated as six replications.

The experimental design included three series of germinations.
(1) All the pollen lots were germinated under routinely used in vitro conditions, i.e. for 27 h at +28°C in the dark (Häggman et al. 1997; Aronen et al. 1998; Venäläinen et al. 1999), in order to study the variation in pollen viability. In the 1998 collection germination was performed with fresh pollen, while in the 1996 collection pollen stored for 18 months at –20°C was used.
(2) In the 1998 germinations the behaviour of 21 pollen lots selected from the seed-orchard genotypes and the five pollen lots originating from the surrounding forests was studied under varying germination conditions, either outdoors or indoors at different constant temperatures. In the outdoor experiment with fresh pollen, the pollen suspensions on the orbital shaker were placed outside under a light shelter to protect the samples from direct sunlight and rain, but subjected to diffuse illumination and natural temperature variations. During the experiment the highest daily temperatures in the shelter were around +15°C and the lowest night temperatures around +2°C, the daily mean temperature being +8°C. Germination was started in the late afternoon and lasted for 92 h, i.e. until the mixture of pollen samples used for monitoring the progress of germination appeared to be well-germinated as evaluated under a microscope. The dependence of pollen viability on the germination temperature was studied using the same 1998 pollen lots stored for 9 months at –20°C. Germination of the stored pollen was performed in the dark at constant temperatures of +28°C, +18°C, and +8°C. The germination times were adjusted so that the pollen lots received the same effective temperature sum in all treatments: germination lasted for 27 h at +28°C, for 42 h at +18°C, and for 95 h at +8°C.
(3) An experiment was also performed to investigate how long pollen keeps its viability unchanged after shedding. For this pur-

pose, the 1998 pollen of four genotypes (P375, P491, P498 and P695) was kept in open isolation bags in a greenhouse and germinated for 27 h at +28°C in the dark 1, 4, 7, 14, 19 and 26 days after pollen collection.

Measurements of pollen-tube length

Random samples of germinated suspensions of pollen were photographed under an Olympus CK2 microscope (magnification 13.2 ×) using an attached SC35 camera. The negatives of the black and white films were enlarged (10 ×) with a photographic enlarger. The lengths of the pollen-tubes were measured from these enlargements using a calliper rule connected to a computer. About 50 pollen grains were measured per replication, the total number of measurements being about 73 800. The possible appearance of a second pollen-tube was also recorded for each pollen grain. The pollen-tube length of non-germinated pollen grains was recorded as zero. This procedure was adopted because the aim was to determine the actual competitive ability of the pollen lot under in vivo conditions in which a non-germinating pollen grain also occupies space in the pollen chamber. Moreover, very slowly germinating and non-viable pollen grains could not yet be distinguished after a 27-h germination. In addition, the diameter of the pollen grains including sacci was measured on a sample of about 300 pollen grains per pollen lot collected in 1998 and stored for 9 months at –20°C.

Statistical analyses

Statistical analyses of the measurements of pollen-tube length were carried out using the replication means as observations. Single-tube lengths were not used because the tubes growing in the same well could have been affected by the same environmental disturbances, e.g. growth of microbial contaminants. The experimental design thus provides six observations for each lot, i.e. pollen collected from one genotype and treatment combination. The analysis of variance and post-hoc tests for the treatment means were performed using the GLM General Factorial procedure, and the calculation of correlation coefficients using the Bivariate Correlations procedure of SPSS Base 8.0 statistical software (SPSS Inc. 1998).

Results

Differences in pollen viability among genotypes

Considerable variation was observed in both the germination percentage and the average tube growth rate of the pollen lots. After 27-h in vitro –germination, the percentage of germinated pollen grains in the pollen lots collected in 1998 and germinated immediately after harvesting varied from 62 to 98, the grand mean being 91 (± SE 0.8). The average pollen-tube length in these pollen lots ranged from 37 to 252 µm, the grand mean being 160 (± 6.0) µm. In the pollen lots collected in 1996 and stored at –20°C for 18 months before germination, the average germination percentage was 72 (± 1.9), varying from 31 to 94%. The pollen-lot means for tube length in the 1996 material varied from 26 to 249 µm, the grand mean being 131 (± 7.9) µm. When only the germinated pollen grains are considered, the grand means for pollen-tube length in the 1998 and 1996 materials were the same, 175 (± 6.2) and 172 (± 7.8) µm, respectively. There was, however, a significant correlation between the germination percentage and the average tube length of the germinated pollen grains in both years, the Pearson r value being 0.270 ($P = 0.028$) in 1998 and 0.670 ($P = 0.000$) in 1996.

Table 1 Analysis of variance for average pollen-tube length after in vitro germination of 1996 and 1998 pollen lots originating from Norway spruce seed orchard no. 170. The 1996 material was stored at –20°C before germination, while the 1998 pollen was fresh

Source	*df*	MS	F-value	*P*-value
Pollen donor	65	25 085	34.1	0.000
Collection year	1	89 360	121.4	0.000
Interaction	64	12 506	17.0	0.000
Error	654	736		

The significance of the factors affecting pollen viability (length measurements including non-germinated pollen grains as zero values), i.e. pollen donor and collection year, was studied using analysis of variance. Both the effects of the pollen donor and collection year, as well as their interaction, were found to be significant ($P = 0.000$) (Table 1). The Student-Newman-Keuls test for a multiple comparison of means also indicated significant ($P<0.05$) differences among the pollen donors. When the collection years were analysed separately, the pollen donors from the area surrounding the seed orchard, i.e. the trees representing background pollinators, did not form a group of their own. Their lot-means fell evenly within the total range of the tube-length values. The Pearson correlation coefficient for the pollen-lot means in tube length between the years 1998 and 1996 was 0.324 ($P = 0.008$).

There was also variation in the average pollen-grain diameter and in the appearance of a second pollen-tube among the pollen lots. The pollen-lot means for the grain diameter varied from 95 to 116 µm, the grand mean being 106 (± 0.98) µm. In 1998, the average percentage of pollen grains with a second tube varied from 0 to 49%, the grand mean being 14 (± 1.3). In the 1996 material, the corresponding values were 2–54% and 21 (± 1.5). The Pearson r value for the pollen-lot means in the ap-

Table 2 Analysis of variance for average pollen-tube length of 1998 pollen lots germinated under varying germination conditions, (a) fresh pollen germinated at a constant temperature of +28°C and outdoors, and (b) stored pollen germinated at +28°C, +18°C, and +8°C

Source	*df*	MS	F-value	*P*-value
a				
Pollen donor	24	11 357	13.9	0.000
Germination condition	1	459 686	562.9	0.000
Interaction	24	6303	7.7	0.000
Error	250	817		
b				
Pollen donor	25	31 241	108.4	0.000
Germination condition	2	299 773	1040.8	0.000
Interaction	50	7352	25.5	0.000
Error	390	288		

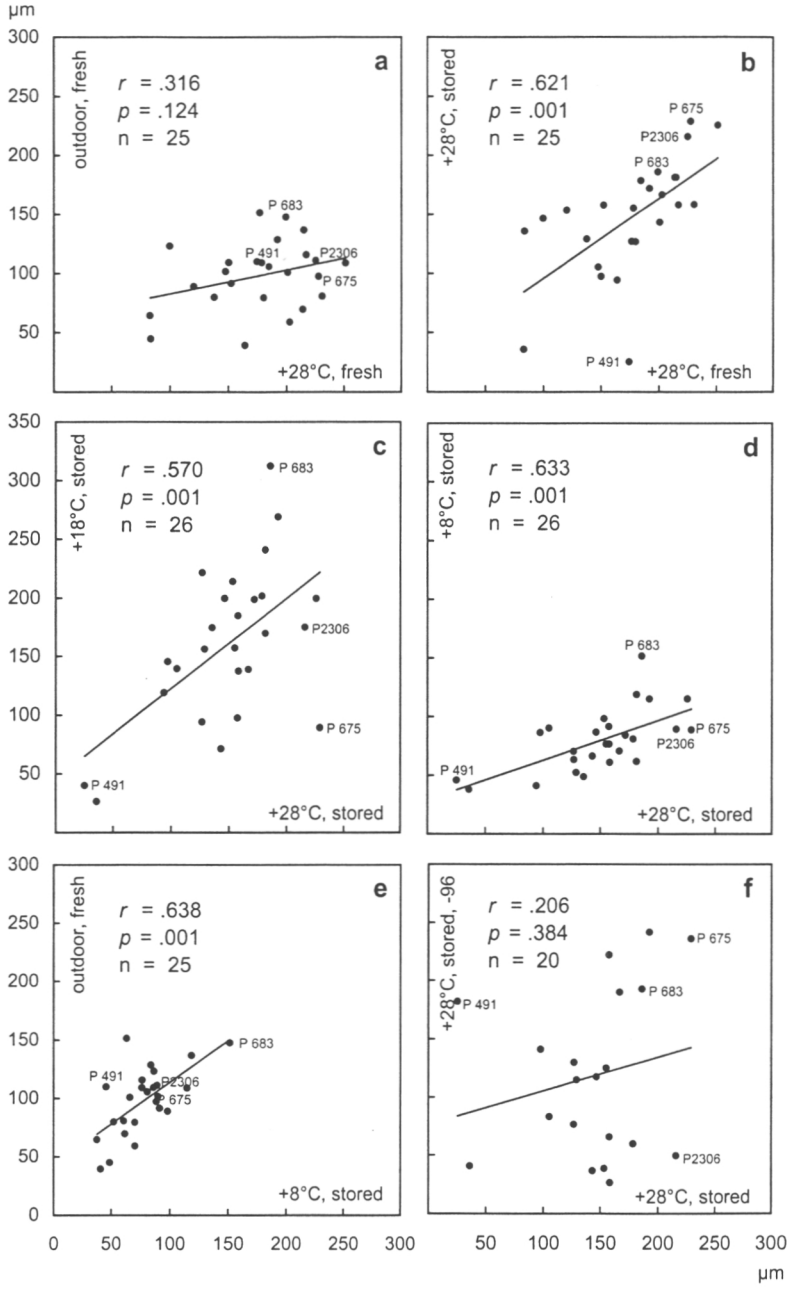

Fig. 2a–f The average pollen-tube lengths after in vitro germination under varying conditions. The Pearson correlation coefficient (r) together with its significance (P) and the number of pollen lots (n) in each comparison are shown. Some of the pollen lots are marked with their clone number. Comparison between **a** germination at a constant temperature of +28°C and outdoors using fresh 1998 pollen, **b** fresh and stored 1998 pollen at +28°C, **c** stored pollen at +28°C and +18°C, **d** stored pollen at +28°C and +8°C, **e** stored pollen at +8°C and fresh pollen germinated outdoors, and **f** stored 1998- and 1996-pollen at +28°C

pearance of a second tube between the years 1998 and 1996 was 0.555 (P = 0.000). Also the correlation between the appearance of the second tube and the germination percentage or the average pollen-tube length was positive and significant in both years: with Pearson correlation coefficients of 0.403 and 0.398 (P = 0.001 for both) in 1998, and 0.470 (P = 0.000) and 0.277 (P = 0.026) in 1996, respectively.

Potential connections between the variation observed in pollen viability and other characteristics of the pollen

donors or specific exogenous factors were studied using correlation analysis. No correlation was found with the geographical origin, the abundance of male flowering, or the average performance of the progenies of the pollen-donor genotype, nor with the growth characteristics or the phenology of male flowering of the particular graft from which the pollen was collected. No correlation was found with the average pollen-grain diameter, and none with graft spacing or soil factors, such as the pH or exchangeable Ca, K, P, or Mg concentrations of the growing site of each particular pollen-donor graft. Also when the appearance of the second pollen-tube was examined, no correlations with any genotype, graft, or soil characteristics studied were found.

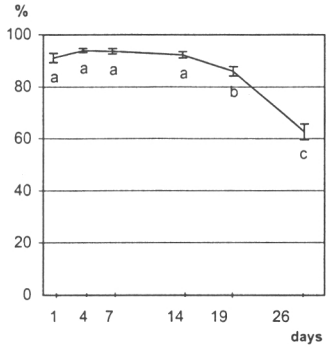

Fig. 3 The mean germination percentage of pollen lots P375, P491, P498, and P695 at 1–26 days after pollen collection. The daily means marked with differing letters differ significantly from each other, $P<0.05$ in the Student-Newman-Keuls test for a multiple comparison of means

Effect of germination conditions on pollen viability

The behaviour of the pollen lots under varying environmental conditions was studied by changing the in vitro germination conditions of the 1998 material. The significance of the pollen donor and germination conditions, as well as their interaction, was studied using analysis of variance. The effects of the pollen donor, germination conditions and their interaction were found to be significant ($P = 0.000$) in the case of both fresh pollen germinated at a constant temperature of +28°C in the dark and outdoors (Table 2a), and for pollen stored at –20°C and germinated at temperatures of +28°C, +18°C and +8°C (Table 2b).

The pollen-lot means for tube length in different germination conditions were compared using correlation analysis. The pollen-lot means of the most important comparisons are plotted against each other in Fig. 2. No significant correlation was found between the pollen-lot means when fresh pollen was germinated either under routinely used in vitro conditions, i.e. for 27 h at +28°C in the dark, or outdoors (Fig. 2a).

Based on the finding that the pollen lots, either fresh or stored at –20°C, behaved in a rather similar fashion under the same germination conditions, with the exception of lot P491 (Fig. 2b), the effect of germination temperature on pollen viability was studied more closely using pollen stored at –20°C. As can be seen from Fig. 2c and d, there was a significant positive correlation between germination at either +18°C or +8°C and at +28°C. When the pollen was germinated at +18°C the differences among the pollen lots were larger than at +28°C, although the grand means for these germinations were similar, 161 (± 13) µm and 149 (± 9.7) µm. At +8°C, all the pollen lots germinated slowly, the grand mean being 80 (± 5.2) µm. The differences among the lots were subsequently smaller. There were a few pollen lots that behaved differently under varying germination conditions. For example, lots P675 and P2306 benefited from a higher germination temperature, while lot P683 germinated relatively faster at lower temperatures. The comparison between germination outdoors under temperatures varying from +2 to 15°C and germination at +8°C showed a significant positive correlation (Fig. 2e) that was also present between germination outdoors and at +18°C, the Pearson r value for the latter case being 0.529 ($P = 0.006$). No correlation was found between the means for pollen-tube length in the lots collected in 1998 and 1996 and stored at –20°C for either 9 or 18 months and germinated under the same conditions (Fig. 2f).

Persistence of pollen-germination ability

The persistence of pollen viability was studied by measuring the germination percentage and tube length of the germinated pollen grains of lots P375, P491, P498, and P695. The germination percentage remained unchanged for 2 weeks after pollen shedding (Fig. 3). The tube length of the germinated pollen grains varied between 164 (± 15) and 214 (± 11) µm during days 1–19, and was significantly lower (138 (± 18) µm, $P<0.05$ in a Student-Newman-Keuls test for a multiple comparison of means) on day 26 after shedding.

Discussion

There was significant variation in pollen viability among the *P. abies* genotypes in the seed orchard, the differences among the pollen-lot means being 7–10–fold in different years. These results confirm the earlier report of considerable variation in pollen-tube growth rate among individuals selected from natural populations of *P. sylvestris* (Venäläinen et al. 1999). There is good evidence that the pollen-tube growth rate of angiosperms is phenotypically variable, and that this variation affects fitness (see Havens 1994). It has been suggested that pollen competition might also be one of the reasons for unequal reproductive success in gymnosperms (Schoen and Cheliak 1987; Nakamura and Wheeler 1992 and ref-

erences therein; Skrøppa and Lindgren 1994). Ours, however, is the first report showing variation in pollen viability in a seed orchard of an industrially important tree species.

In the present work, differences in pollen viability were studied in vitro. Germination and the early stages of the tube growth of spruce pollen have been found to be similar both in vitro and in vivo, although they occur faster in the former (Dawkins and Owens 1993; Martinussen 1994; Lazzaro 1996). After 27-h in vitro germination in the present study, the pollen-tubes in the fastest lots had elongated on the average to 250 µm. This is approximately 1/3 of the distance they have to grow in a female flower to reach the archegonium and fertilise the egg cell (Sarvas 1968; Christiansen 1972). In the slowest pollen lots the tubes had elongated on the average to only about 30 µm, i.e. approximately 1/25 of the total distance in the female flower. In nature, tube formation in *P. abies* pollen takes place during two growth periods, interrupted by a resting period, during which both male gametes and archegonia with egg cells develop. In the first growth period, which occurs soon after pollination, the tube attains a length of about 240–400 µm and then continues to grow 3–4 weeks later, i.e. immediately before fertilisation (Sarvas 1968; Christiansen 1972).

The reproductive biology of *P. abies* provides an opportunity for male gametophyte competition through differential germination ability and tube growth rate. The pollen chambers of the species can accommodate more than ten pollen grains, five being the average number (Sarvas 1968). Fertilisation takes place relatively soon after pollination and, under in vitro conditions at least, elongation of the tubes continues in a linear fashion up to lengths comparable with the final distance to the archegonia (Martinussen 1994; Martinussen et al. 1994). This suggests that the resting period reported during in vivo germination may not change the order of competing tubes. Moreover, no pre-zygotic incompatibility mechanisms have been reported. However, there are also features that might affect the results of gametophyte competition. In *P. abies*, there is usually more than one archegonium per ovule, and in most cases two competing embryos are formed to ensure the formation of full seed. Thus the genotypes homozygous for lethal, sublethal or defective genes are eliminated either by early abortion of the zygotes or, later on, through embryo competition (Sarvas 1968).

According to the results of the present study, the fitness of the *P. abies* pollen donors with respect to the germination ability and tube length of their pollen varies, as has also been reported in many angiosperm species. According to Havens (1994), however, it is unclear whether the variation found in pollen performance is really heritable or is caused by environmental effects. Her results obtained with pollen from cuttings of *Oenothera organensis* suggest that the condition of the plant, flowering shoot and / or flower may be more important than genotype in determining the pollen-tube growth rate. In the present study, only a single graft per genotype was used as pollen donor but, on the other hand, potential connections between pollen viability and several exogenous factors and other characteristics of the pollen donors were also investigated. Since no correlation was found between the average pollen-tube length and, e.g., the phenology, growth or site characteristics of the pollen donors, the variation found is expected to reflect the genetic potential of the genotypes. Unlike several studies on angiosperms (Mulcahy 1983 and references therein; Quesada et al. 1993), no connection was, however, found between the pollen-tube growth rate and the performance of the progenies. This is in accordance with the results obtained with another conifer, *P. sylvestris* (Venäläinen et al. 1999).

Significant genotype-year interaction was found when the pollen viability of all the genotypes of the seed orchard were examined in different collection years. The varying behaviour of the pollen lots in the 1996 and 1998 collections may be caused by a number of factors. The weather conditions in 1996 and 1998 during flowering were different. The fact that the 1996 material was stored at –20°C before germination complicates the results because some of the pollen lots may have suffered from freezing, as was found for the 26 lots in the 1998 material that were germinated under varying conditions.

Our study suggests that there are pollen lots that either benefit from a higher germination temperature or germinate faster at lower temperatures. This may indicate the adaptation of different *P. abies* genotypes to produce fast-germinating pollen for different environmental conditions. As pointed out by Delph et al. (1997), genotype-environment interactions in pollen performance will promote the maintenance of genetic variation within populations even if pollen performance is related to fitness. Johnsen et al. (1996) had earlier suggested that some environmental signals during the reproductive process taking place in the female flowers, for instance pollen-tube growth, may cause variation in the phenology traits of the progeny. In seed orchards, these phenomena may contribute to the variable genetic composition of seed produced in different years.

As a consequence of the genotype-environment interactions in pollen performance, a single in vitro germination under routinely used conditions cannot give a complete picture of the variation among genotypes. According to the literature, the recommended conditions for the in vitro germination of spruce pollen include a relatively high constant temperature, from +25 to +30°C, and no illumination (Christiansen 1972; Lanteri et al. 1993; Martinussen 1994; Lazzaro 1996; Häggman et al. 1997; Aronen et al. 1998). In the present study the lot-means for pollen viability at a constant temperature of +28°C did not correlate with those in outdoor conditions, while the correlation between pollen viability at a constant temperature of +8°C and outdoor conditions was positive and significant. On the other hand, a significant positive correlation for pollen viability was found when the germination temperature alone was changed. In future studies, however, it would be reasonable to use lower in vitro

germination temperatures than the recommended temperature of +25 to +30°C in order to achieve the results that best correspond to the variation occurring in nature. The weather conditions in Finland during the flowering period of spruce differ considerably from year to year, the daily mean temperatures varying from +8 to +21°C during 1984–95, with a mean of +13°C (Nikkanen and Ruotsalainen, unpublished). The outdoor conditions in 1998 were colder than the average but still favourable for successful pollination in *P. abies*.

Under the experimental conditions employed in this study, i.e. dry pollen in open isolation bags in the greenhouse, both the germination percentage and the tube growth of *P. abies* pollen remained unchanged for 2 weeks after pollen shedding. Hak (1996) has earlier shown that vacuum-processed *Picea mariana* pollen stored for 1 year at +18°C can retain a high germinability of 78%. In the case of *P. abies* and *P. sylvestris*, it has been estimated that the share of pollen that has migrated from another population located over hundreds of kilometres away is small on the average, but can account for a significant proportion of the total pollination in some years (Koski 1970; Lindgren et al. 1995). Moreover, long-distance pollen of *P. sylvestris* has been shown to maintain high germinability (Lindgren et al. 1995). In spruce seed orchards, the high persistence of the germination ability of pollen enables fertilisation by pollen that has travelled over long distances. Air-borne pollen is exposed to direct ultraviolet radiation, and this has been shown to reduce pollen-tube growth in 19 of 34 taxa representing both monocotyledons and dicotyledons. In coniferous pollen both stimulating and inhibiting effects have been reported (Torabinejad et al. 1998 and references therein).

The significant variation in pollen viability found among the *P. abies* genotypes in the present seed orchard study indicates a potential for male gametophyte competition. Together with the observed genotype-environment interactions in pollen performance, this may contribute to the variable genetic composition of the seed produced in the orchard. The magnitude of the effects of pollen viability can, however, be assumed to be smaller than the effects of variation in flowering abundance and phenology within the seed orchard and of pollination by non-orchard sources. Pollen-tube competition in *P. abies* will be further studied by making controlled crossings with pollen-lot mixtures including fast and slowly elongating pollen-tubes, and by carrying out paternity analysis on the progenies.

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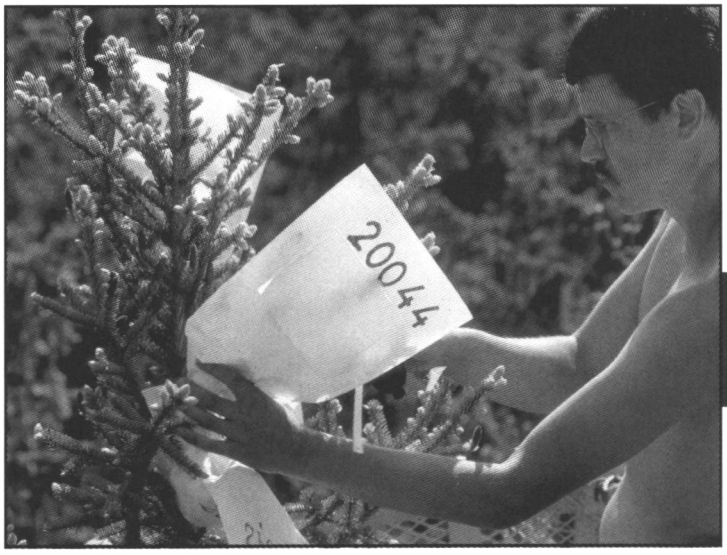

IV

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Pollen competition and seed-siring success in *Picea abies*

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Abstract The aim of the present work was to study pollen-tube competition in *Picea abies*. Controlled crossings were performed with pollen mixtures including pairs of pollen lots with fast and slowly elongating pollen-tubes. Paternity analysis using isozyme markers was performed on the progenies in order to study whether the in vitro pollen-germination vigour corresponds to the proportion of seeds sired by the pollen donor. Paternal success was found to be unequal, 15 out of 23 crossings producing progeny that differed significantly from the hypothetical ratio of 1:1. The paternal contribution in the majority of the crossings was as expected: the pollen parent with more-vigorous in vitro germination sired more seeds than the less-vigorous pollen. In the case of two pollen mixtures, however, the seed-siring success summed over the maternal trees was the opposite to the expected value. Despite these aberrations, the results support the hypothesis that pollen-tube competition is one of the factors contributing to male fitness in *P. abies*. However, when all the other factors affecting pollination and seed set under natural conditions are taken into account, it is clear that the seed-siring success of a particular paternal genotype cannot be predicted reliably by measuring only the in vitro pollen vigour.

Keywords Norway spruce · Pollination · Germination of pollen · Pollen-tube growth · Paternal success

Introduction

In many forest-tree species, including Norway spruce (*Picea abies*), the genetic gain achieved by breeding is transferred to production populations through sexual reproduction in clonal seed orchards consisting of genetically superior individuals. As a result of random mating within a seed orchard, the genetic composition of the seed crop should be close to that of the original clones. However, deviations from random mating within a seed orchard are an established fact. There are large differences in flowering abundance among clones and between years (Sweet 1975; Lindgren et al. 1977; Skrøppa and Tutturen 1985; Nikkanen and Ruotsalainen 2000), as well as variation in reproductive phenology (Blush et al. 1993; Nikkanen 2001). In addition, owing to the effective pollen dispersal of this species, pollen contamination from non-orchard sources has also proved to be a serious problem for the proper functioning of seed orchards (Savolainen 1991; Paule et al. 1993; Pakkanen et al. 2000).

Male fitness depends not only on the flowering traits mentioned above, but also on the traits of a pollen grain, such as germination vigour, germination time, pollen-tube growth rate and selective fertilisation (Pfahler 1975). In Norway spruce there is no evidence that maternal plants could control the pollen-tube growth rate, as suggested for other species by Hormaza and Herrero (1996).

Competition among male gametophytes has been extensively studied and discussed in angiosperms (Mulcahy 1983 and references therein; Charlesworth 1988; Quesada et al. 1993). In a deciduous tree species, *Betula pendula* (Pasonen et al. 1999), and in a perennial marsh plant, *Hibiscus moscheutos* (Snow and Spira 1991, 1996), a faster pollen-tube growth rate of the pollen donor was associated with the siring of a larger number of seeds. The rankings of the pollen donors were consistent across the different maternal plants. Furthermore, in several gymnosperm species, e.g. *Pseudotsuga menziesii, Pinus radiata, Pinus taeda* and *P. abies*, the application of pollen mixtures has resulted in unequal paternal success of the pollen donors. Pollen competition, including different rates of germination and pollen-tube growth, has been suggested as one of the reasons for this phenomenon (Schoen and Cheliak 1987;

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Table 1 The pollen mixtures and isoenzyme loci used for paternity analysis. For isoenzyme genotypes, F=fast and S=slow allele

Pollen mixture	Sire clones	Isoenzyme locus studied	Isoenzyme genotype
Mixture 1	P491	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF
	P1203		SS
Mixture 2	P498	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF
	P1203		SS
Mixture 3	P491	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF
	P1203		SS
Mixture 4	P498	DIA, diaphorase, E.C. 1.6.4.3, slower locus	FF
	P1203		SS
Mixture 5	P675	GDH, glutamate dehydrogenase, E.C. 1.4.1.2	FF
	P695		SS

Nakamura and Wheeler 1992; Skrøppa and Lindgren 1994). In previous reports, we have shown differences in the average in vitro pollen-tube growth rate among pollen donors of *Pinus sylvestris* (Venäläinen et al. 1999) and *P. abies* (Nikkanen et al. 2000). However, it is not known whether the variation in this trait also affects the genetic composition of the seed produced in seed orchards.

The aim of the present work was to study pollen-tube competition in *P. abies* in more detail. Controlled crossings were performed with pollen mixtures including pairs of pollen lots with fast and slowly elongating pollen-tubes. Paternity analysis was performed on the progenies obtained in order to study whether the in vitro pollen germination vigour corresponds to the proportion of seeds sired by the pollen donor.

Materials and methods

Pollen from 66 clones was collected from *P. abies* seed orchard no. 170 (Heinämäki), located at Korpilahti, southern Finland (62°13′N, 25°24′E). Pollen grains were germinated in vitro under routinely used conditions, i.e. for 27 h at 28°C in the dark, and germination vigour was measured as described by Nikkanen et al. (2000). Pollen lots from each tail of the distribution, i.e. those showing either fast or slow tube-elongation, were considered as candidates for controlled pollinations. Based on distinguishable isoenzyme genotypes, lots P491, P498, P675, P695 and P1203 were selected for the pollen mixtures.

Five pollen mixtures were used to pollinate five seed parents (P495, P683, P689, P1206, P2579, three grafts/clone) in 1998. Each of the mixtures consisted of an equal mass of pollen from two clones. On the basis of the in vitro germination results of frozen pollen from 1996 (Nikkanen et al. 2000), one clone was supposed to have poor and the other one good in vitro pollen germination vigour. Mixtures 1 and 2 consisted of pollen collected in 1996 and stored at –20°C for 2 years, while mixtures 3, 4 and 5 comprised pollen collected just before the controlled pollinations were performed. The pollen donors in mixtures 3 and 4 were the same as those in mixtures 1 and 2 (Table 1).

The pairs of sires for controlled pollination were composed on the basis of identifiable isoenzyme genotypes. Seed paternity was determined by genotyping germinated embryos at two enzyme loci, one for each combination (Table 1). The method described for example by Muona et al. (1987, 1990) was followed. For homozygotic seed parents we needed to analyse only embryos, but for heterozygous seed parents the megagametophytes also had to be studied in order to infer the paternity (Table 2).

Table 2 Isoenzyme genotypes of the seed parents at the loci studied. For isoenzyme genotypes, F=fast and S=slow allele

Seed parent	Isoenzyme locus	
	DIA	GDH
P495	SS	FS
P683	FF	FF
P689	FS	FS
P1206	SS	FF
P2579	FS	FF

The null hypothesis of the equal contribution of the sires to the germinating seeds was examined by using a chi-square test of the goodness of fit (Sokal and Rohlf 1995).

Results

Paternity analysis showed a discrepancy in the equal paternal contribution in the germinating seeds from all the pollen mixtures (Tables 3 and 4). Table 3 shows the paternal contribution in the germinated seeds expressed as a ratio, in which the count of offspring from the more-vigorous pollen parent was divided by the count of offspring from the less-vigorous pollen parent. Since the measure for the more-vigorous sire in vitro was the numerator, all the values of the ratio would be greater than one if the outcome was in the same direction as that in the in vitro test. Of the 23 ratio values for the five pollen mixtures and five seed parents (two seed lots were missing), 15 were greater than one and eight were less than one (Table 3). The chi-square test for the goodness of fit for the 1:1 ratio of the paternal contribution showed that 15 out of the 23 ratio values differed significantly from the hypothetical 1:1 ratio (Table 4). Four of the statistically significant ratio values were in an opposite direction to that expected, and hence the less-vigorous sire in vitro had more offspring than the more-vigorous one.

Crossings with two pollen mixtures were performed using both fresh (mixtures 3 and 4) and old pollen stored at –20°C (mixtures 1 and 2). As shown in Table 3, the ratios of pollen-tube lengths in the mixtures of fresh

Table 3 Paternal success in controlled pollinations of *P. abies*. All the ratio values are expressed in the order in which the pollen parent with faster growing pollen-tubes are in the numerator, and the more slowly growing in the denominator. The ratio values which differed significantly from the 1:1 ratio are marked in bold

Pollen mixture, pollen donors	Ratio of pollen-tube length	Ratio of paternal success Seed parent					
		P495	P683	P689	P1206	P2579	Together
Mix 1 P491/P1203	6.93	0.94	**2.88**	0.58	**0.48**	**0.20**	**0.71**
Mix 2 P498/P1203	8.47	0.87	**2.09**	**2.00**	1.36	2.11	**1.61**
Mix 3 P1203/P491	1.32	**7.25**	**3.57**	**3.71**	**8.50**	**16.00**	**5.86**
Mix 4 P1203/P498	1.51	**2.00**	**2.10**	1.06	–	**2.20**	**1.75**
Mix 5 P675/P695	2.74	**0.39**	**0.39**	1.24	–	0.52	**0.60**

Table 4 Chi-square test for goodness of fit for the 1:1 ratio of the paternal contribution estimated from the germinated seeds. Statistically significant deviations from the 1:1 ratio are marked in bold

Seed parent		Pollen mixture				
		Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5
P495	*n*	31	28	33	36	32
	χ^2	0.03	0.14	**18.94**	**4.00**	**6.12**
	p	0.9>*p*>0.5	0.9>*p*>0.5	*p*<0.001	0.05>*p*>0.025	0.025>*p*>0.01
P683	*n*	31	34	32	31	32
	χ^2	**7.26**	**4.23**	**10.12**	**3.90**	**6.12**
	p	0.01>*p*>0.005	0.05>*p*>0.025	0.005>*p*>0.001	0.05>*p*>0.025	0.025>*p*>0.01
P689	*n*	30	36	33	33	38
	χ^2	2.13	**4.00**	**10.94**	0.03	0.42
	p	0.5>*p*>0.1	0.05>*p*>0.025	*p*<0.001	0.9>*p*>0.5	0.9>*p*>0.5
P1206	*n*	43	33	19	–	–
	χ^2	**4.23**	0.76	**11.84**	–	–
	p	0.05>*p*>0.025	0.5>*p*>0.1	*p*<0.001	–	–
P2579	*n*	30	28	34	32	32
	χ^2	**13.33**	3.57	**26.47**	**4.50**	3.12
	p	*p*<0.001	0.1>*p*>0.05	*p*<0.001	0.05>*p*>0.025	0.1>*p*>0.05
Sum over mothers	*n*	156	159	151	132	134
	χ^2	**4.33**	**8.61**	**75.82**	**9.82**	**8.63**
	p	0.05>*p*>0.025	0.005>*p*>0.001	*p*<0.001	0.005>*p*>0.001	0.005>*p*>0.001

pollen differed considerably from the ratios obtained for stored pollen. When comparing paternal success between mixtures of freshly collected and frozen pollen, in mixtures 1 and 3 pollen lot P1203 had a better paternal success than lot P491, independently of the ratio value for tube lengths: in seven out of ten crossings this lot sired significantly more progenies than P491. By contrast, in mixtures 2 and 4 the sire with longer pollen-tubes in vitro sired more seeds.

When the contribution of each pollen mixture was summed over the seed parents, three ratio values out of five were in agreement with the in vitro tests (Table 3). All the ratio values deviated significantly from 1:1.

Discussion

In the present study, paternal success in *P. abies* proved to be unequal, 15 of the 23 crossings producing progeny that differed significantly from the hypothetical 1:1 ratio. Similar results have been observed previously not only in *P. abies* (Schoen and Cheliak 1987; Skrøppa and Lindgren 1994) but also in some other gymnosperms such as *P. menziesii*, *P. radiata* and *P. taeda* (Nakamura and Wheeler 1992 and references therein).

The paternal contribution in the majority of the present crossings was as expected: the pollen parent that germinated more vigorously in vitro sired more seeds than the less-vigorous pollen. A similar connection between pollen-tube growth rate and parental success has previously been reported in a number of angiosperm species (Snow and Spira 1991, 1996; Pasonen et al. 1999). The present result is in accordance with the reproductive biology of *P. abies*, which provides an opportunity for male gametophyte competition. The pollen chambers of the species can accommodate more than ten pollen grains, five being the average number (Sarvas 1968). Pollen-tube formation takes place during two periods, interrupted by a resting period, resulting in fertilisation 3–4 weeks after pollination (Sarvas 1968; Christiansen 1972). Under in vitro conditions, elongation of the tubes is linear up to lengths comparable with the final distance

to the archegonia (Martinussen 1994; Martinussen et al. 1994), suggesting that the in vivo resting period may not change the order of competing tubes. Moreover, no prezygotic incompatibility mechanisms have been reported.

In the case of two of the pollen mixtures, 1 and 5, the seed siring success summed over the seed parents was the opposite to the expected one. There are several probable reasons for this. As observed in our previous study (Nikkanen et al. 2000), in vitro germination under a relatively high constant temperature does not give a complete picture of the whole variation in the germination potential of *P. abies* pollen lots. Generally, the ranking of the pollen lots remains relatively stable when the germination temperature is changed, or when stored pollen is used instead of freshly collected pollen. There are, however, pollen lots that behave differently under varying germination conditions. For example, lot P675 included in mixture 5 benefits from a higher temperature (Nikkanen et al. 2000). Thus its poor parental success in the present study could be at least partly explained by the fact that the flowering period in 1998 was colder than the long-term average (Nikkanen and Ruotsalainen 2000). On the other hand, pollen lot P1203, which was included in mixtures 1 and 3, was found to have varying vigour in different years. According to Havens (1994) and Delph et al. (1997), variation in pollen performance can be partly caused by environmental effects, such as temperature or the physiological condition of the donor plant during pollen development. The fact that the pollen in mixtures 1 and 2 was stored at –20°C before germination may also affect the results, because some pollen lots seem to suffer from freezing (Nikkanen et al. 2000). However, lot P1203 sired more seeds than its competitor, P491, irrespective of its in vitro germination result.

The reproductive biology of the species includes features that might change the outcome of gametophyte competition. In *P. abies* there is usually more than one archegonium per ovule, and in most cases two competing embryos are formed in order to ensure the formation of full seed. Thus the genotypes homozygous for lethal, sublethal or defective genes are eliminated either by early abortion of the zygotes or, later on, through embryo competition (Sarvas 1968). In the present study, however, there was no indication that these features would have been more active in any of the parental combinations than in the others.

According to Pasonen (2000), in an angiosperm tree, *Betula pendula,* genotype-environment interactions were found in pollen-tube growth rate and seed-siring success, but the changes in the rankings of the pollen donors did not translate into parallel changes in seed-siring success. In greenhouse conditions, the tube growth rate controlled the paternity of the birch seeds, but in the more heterogeneous outdoor environment a negative correlation was found between pollen elongation and paternal success. This result was assumed to be due to differences in the physiological condition of the maternal plants, caused by microhabitat variation. Since the maternal plant is known to provide carbohydrates for germinating pollen also in gymnosperms (Willemse and Linskens 1969; Johri 1992; Dawkins and Owens 1993), the same type of natural environmental variation could have affected the pollen competition in the present study.

Despite some aberrations, the present results support the hypothesis that pollen-tube competition is one of the factors contributing to male fitness in *P. abies*. There are genotype-environment interactions in pollen performance, as shown already by Nikkanen et al. (2000) and also reflected in the present study, that affect the paternal success. These G×E interactions promote the maintenance of genetic variation within populations, even if pollen performance is related to fitness (Delph et al. 1997); and in seed orchards they may contribute to the variable genetic composition of the seed produced in different years. On the basis of the present results, and taking into consideration all the other factors affecting pollination and seed set under natural conditions, it would appear that the seed-siring success of a particular paternal genotype cannot be predicted reliably by measuring the in vitro pollen vigour only.

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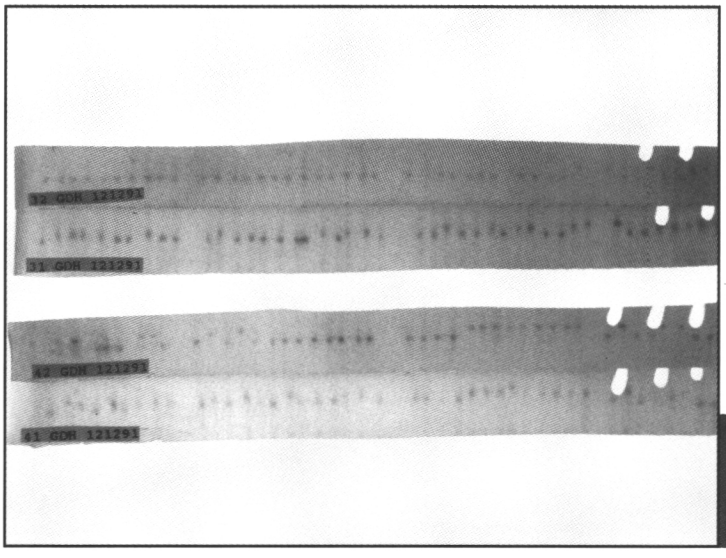

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Annual Variation in Pollen Contamination and Outcrossing in a *Picea abies* Seed Orchard

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A mature Norway spruce [*Picea abies* (L.) Karst.] seed orchard, established in southern Finland with 67 clones from northern Finland, was analysed in three different years in order to estimate the pollen contamination ratios. Allozyme-based paternity analysis revealed that the contamination rate was high, 69–71%, and did not differ between the years studied. It appears that, in areas where spruce is the dominant tree species, the contamination rate will be very high even in mature seed orchards. However, the contamination rate in the thinned parts of the orchard was significantly lower than that in the unthinned parts in two of the three years studied. The outcrossing rate was also high, 96–100% in all years, even though the ramets of each clone were planted using a clonal-row design, and there were no significant differences in the outcrossing rates between the different parts of the orchard. *Key words: allozymes, background pollination, mating system, Norway spruce, selfing.*

INTRODUCTION

The annual variation in female flowering and seed crop in Norway spruce [*Picea abies* (L.) Karst.] is large (Heikinheimo 1932, Sarvas 1968, Koski & Tallqvist 1978). In southern Finland there are usually no more than two or three good flowering years each decade, and in northern Finland even less frequently. Furthermore, the seed production capacity seems to decrease on moving towards the harsh conditions in the north (Sarvas 1968). The year-to-year variation in pollen production is smaller than that in seed production (Koski & Tallqvist 1978, Ruotsalainen & Nikkanen 1989). Anthesis also seems to be poorly adapted to the northern conditions in areas where the average effective temperature sum is less than 1000 degree days (dd) (Luomajoki 1993).

In order to ensure a supply of reforestation material for northern Finland, seed orchards of Norway spruce and Scots pine (*Pinus sylvestris* L.) have been established in southern Finland. During 1968–1972 six Norway spruce seed orchards, totalling 99 ha in size and comprising clones from latitudes 64–68°N, were established in areas between latitudes 61 and 62°N. It was expected that the orchard clones, moved from north to south, would be pollinated by each other before the shedding of local pollen. This assumption was based on the theory that the temperature criterion for the onset of flowering would be smaller in trees adapted to northern conditions with short and cool summers than in those adapted to more southern conditions (Sarvas 1962, 1968, 1970). In Scots pine this is the case with female flowers, but male flowering tends not to start much earlier than that in local southern forests (Pulkkinen 1994). The time difference between the onset of female and male flowering within Scots pine orchards may even be several days (Pulkkinen 1994), thus increasing the risk of non-orchard pollination.

Even in mature Scots pine seed orchards with northern clones transferred to the south, and with high pollen production, the estimated pollen contamination rates are relatively high, ranging from 45 to 76% (Pakkanen & Pulkkinen 1991), from 51 to 58% (Wang et al. 1991) and from 69 to 74% (Yazdani & Lindgren 1991). As a result of pollen contamination from origins located further to the south than the seed orchard clones, the offspring of Scots pine are not adapted to the intended utilization area (Nikkanen 1982, Pulkkinen et al. 1995).

Multilocus allozyme markers and paternity analysis have been used to estimate pollen contamination and the mating system in a number of conifer species

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(e.g. Friedman & Adams 1985*a*, 1985*b*, Ritland & El-Kassaby 1985, El-Kassaby et al. 1989, Harju & Muona 1989, El-Kassaby 1997), including Norway spruce seed orchards (Paule et al. 1993). To the authors' knowledge, however, no work has yet been carried out on variation in the contamination rate among years in Norway spruce seed orchards.

The objective of the present study was to estimate the pollen contamination rate in three different years in a Norway spruce seed orchard established with clones moved 2–5° latitude southwards. The proportion of outcrossing was also estimated in a seed orchard established using a clonal-row design. The within-orchard differences in the rate of pollen contamination and the level of outcrossing were also studied.

MATERIALS AND METHODS

The material for this study was obtained from clonal Norway spruce seed orchard no. 170, established in 1968 at Heinämäki in southern Finland (62°13′ N, 25°24′ E) with an effective temperature sum of 1100 dd (5°C threshold). The orchard is situated on a hill, 160–190 m a.s.l., and is surrounded by spruce forests. The area of the orchard is 13.2 ha.

The seed orchard consists of 67 clones originating from between latitudes 64 and 67° N in northern Finland (Fig. 1). The effective temperature sum at the clone origin varies between 820 and 1070 dd, with an average of 888 dd. The grafts were planted using a spacing of 3.5 × 6.5 m and arranged in a clonal-row design, the grafts of the same clone being located 6.5 m from each other. In 1987, half of the orchard (blocks II and IV) was thinned systematically by removing every third graft, and the other half (blocks I and III) was left unthinned (Fig. 2). After thinning, the total number of grafts was 3162.

The seed material for the study was collected in 1989, 1992 and 1993. Cones were collected from one graft per clone in each block. The cones were dried at room temperature, after which the seeds were extracted, cleaned and kept at 4°C until analysis. A total of 1233–2013 seeds was analysed each year (Table 1).

The parental multilocus genotypes were inferred from the allozyme patterns in haploid megagametophyte tissue. Before analysis, the seeds were stratified for 24 h at 4°C, followed by germination for 6 days at room temperature. The multilocus genotypes of the embryos and megagametophytes were assessed at the following 11 allozyme loci: acid phosphatase (E.C.3.1.3.2.), aconitase (E.C.4.2.1.3.), two loci of 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.), 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). Multilocus genotypes that included missing data were not used in the analyses.

The pollen contamination rates were estimated for the whole seed orchard, and separately for the thinned and unthinned parts of the seed orchard. The multilocus genotypes of pollen gametes originating from open pollination were deduced by comparing the allozyme patterns in the megagametophytes and

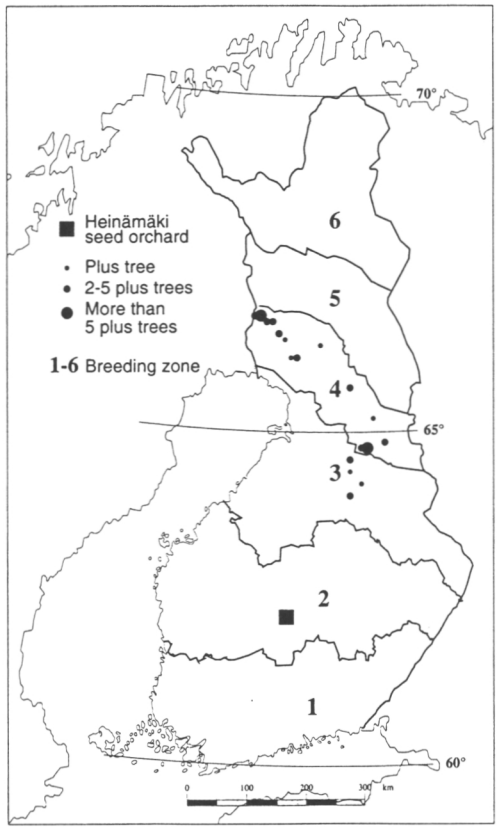

Fig. 1. Location of the Heinämäki seed orchard and the origin of the clones.

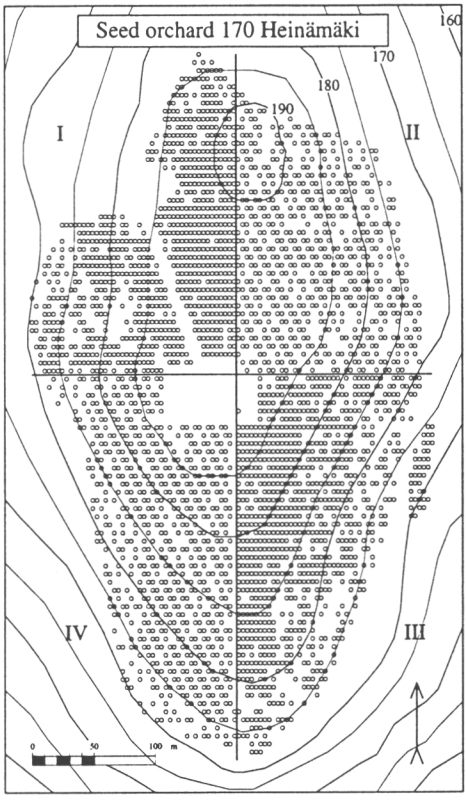

Fig. 2. The Heinämäki seed orchard is situated on a hill. Half of the orchard (blocks II and IV) was thinned in 1987. The grafts are labelled as open circles.

in the corresponding embryos. Pollen gametes with genotypes that could not have been produced by any of the seed orchard clones were regarded as observed contamination (b) from external pollen sources.

Contaminating gametes with genotypes matching those produced by the seed orchard clones could not be identified. If the gene frequencies in the contaminating pollen cloud are known, an estimate of the detection probability of alien pollen (d) can be calculated (Smith & Adams 1983) and used to obtain the adjusted contamination estimate. In this study, the gene frequencies of 400 embryos collected from 99 trees (three to five seeds from each tree) in a Norway spruce stand located 500–1000m south-east of the Heinämäki seed orchard were used instead of the unknown pollen cloud gene frequencies. The estimates of pollen contamination (m) were calculated by the multilocus method described by Smith & Adams (1983) as $m = b/d$. The single locus allele frequencies are used for estimating the frequency of indistinguishable multilocus pollen gamete genotypes (Smith & Adams 1983). The formula for the variance estimate of contamination is given by Friedman & Adams (1985*b*).

The proportions of outcrossing (t) were estimated by the multilocus method of Shaw et al. (1981) for the whole seed orchard, and in one year also for the thinned and unthinned areas separately. Pollen gametes with genotypes that could not have been produced by the mother clone were regarded as outcrossings. The estimated outcrossing rates were obtained by correcting the detected outcrossing rates with the probability of detecting inbreeding, which was estimated by means of the gene frequencies of the seed orchard. The proportion of inbreeding (s) is calculated as $s = 1 - t$.

Differences in the estimated number of pollen contaminants and in the observed number of inbreds between years and between thinned and unthinned areas were analysed using the Pearson χ^2-test.

RESULTS

The observed proportions of alien pollen were 0.076, 0.076 and 0.079 in 1989, 1992 and 1993, respectively, and the probability of detecting alien pollen was 0.110. The estimated pollen contamination rate in the seed orchard was 0.69, 0.69 and 0.71, respectively (Table 1). There were no significant differences in

Table 1. *Seed year, number of analysed seeds, estimated pollen contamination and outcrossing rates and their standard deviations (SD) in the Heinämäki seed orchard*

Seed year	Seed number	Estimated pollen contamination	SD	Estimated outcrossing	SD
1989	2013	0.686	0.053	1.000	0.006
1992	1430	0.693	0.064	0.962	0.009
1993	1233	0.712	0.069	1.000	0.009
Mean	1559	0.697		0.987	

Table 2. *Seed year, number of analysed seeds and estimated pollen contamination rates in the thinned and unthinned parts of the Heinämäki seed orchard (SD in parentheses) and significance level of differences between the thinned and unthinned areas (χ^2-test)*

	Thinned parts		Unthinned parts		
Year	Seed number	Pollen contamination	Seed number	Pollen contamination	χ^2
1989	969	0.550 (0.069)	1044	0.812 (0.080)	159.0***
1992	600	0.680 (0.097)	830	0.702 (0.084)	0.735^{NS}
1993	610	0.653 (0.095)	623	0.770 (0.101)	20.40***
Mean	726	0.628	832	0.761	2.69**

*** $p<0.001$, ** $p<0.01$, $^{NS}p>0.05$.

pollen contamination between the years ($\chi^2 = 0.74$, $p > 0.05$).

The estimated proportions of contaminant male gametes in seed lots collected from the thinned parts of the orchard were 0.55, 0.68 and 0.65, and from the unthinned parts 0.81, 0.70 and 0.77, respectively, in the three years (Table 2). In 1989 and 1993, the proportion of alien pollen was significantly lower in the thinned than in the unthinned parts of the orchard. However, there was no difference between the differently treated parts of the orchard in 1992.

The estimated outcrossing rate for the whole orchard area was 0.96 in 1992 and 1.00 in 1989 and 1993 (Table 1). In 1992, the proportion of inbred seeds in the thinned part (s_1) of the seed orchard was 0.03 and in the unthinned part (s_2) 0.01. The acceptation of the null hypothesis $s_1 = s_2$ ($\chi^2 = 1.49$, $p > 0.05$) indicates that there was no difference in the inbreeding rate between the thinned and unthinned parts. In 1989, 1992 and 1993, the total detected outcrossings were 0.89, 0.93 and 0.92, respectively.

DISCUSSION

The estimated proportion of pollen contamination in the Heinämäki seed orchard was very high, about 70% in each of the years studied, even though pollen production in the seed orchard was abundant in the years when the seed was collected (Nikkanen & Ruotsalainen 2000). Norway spruce is the dominant tree species on 25% of the forest land in Finland, and even higher in southern Finland, 33% (Sevola 1999, Table 1.12). It therefore appears impossible to avoid pollen contamination anywhere in the country. In the region where the Heinämäki seed orchard is located, Norway spruce forests are predominant. The concentration of airborne spruce pollen is thus high during flowering time, increasing the possibility of pollen contamination.

Certain features in the flowering phenology of the seed orchard grafts moved to the south also tend to increase the potential rate of pollen contamination. In 1989, the female flowers in the orchard grafts became receptive 3–5 days before pollen shedding, whereas in 1992 and 1993 the time difference between female receptivity and pollen shedding was 1–3 days (Nikkanen 1993 and unpubl.). However, some pollen was in the air at the time of the onset of female flowering (Nikkanen unpubl.), which must have originated mainly from outside the seed orchard.

The pollen contamination rates reported from southward-transferred Scots pine seed orchards have been at same level as those found at Heinämäki in the present study (Pakkanen & Pulkkinen 1991, Pakkanen et al. 1991, Yazdani & Lindgren 1991). In contrast, Paule et al. (1993) found somewhat lower pollen contamination rates of 0.43–0.59 in two Swedish Norway spruce seed orchards. These Norway spruce orchards, however, had a more northerly location than the present orchard, with a shorter transfer of the seed orchard clones to the south. The synchronization between female and male flowering, and also the isolation from surrounding Norway spruce stands, may have been better in Sweden than at Heinämäki.

The pollen contamination rates in different years were surprisingly similar, even though there were differences between years in flowering phenology and in weather conditions (Nikkanen, unpubl.). Annual differences in pollen contamination have been reported in Scots pine seed orchards (Harju & Muona 1989, Pakkanen & Pulkkinen 1991). Although the present study found no differences in the contamination levels between the three years, in exceptional years some variation may still occur in background pollination in Norway spruce seed orchards.

The Heinämäki seed orchard was capable of producing a large number of different gametotypes, totalling 765, and the probability of detecting alien pollen was rather low. The estimate of pollen contamination rate and its variance estimate are very sensitive to the detection probability (Smith & Adams 1983). The observed pollen contamination rates must be divided by the detecting probability in order to achieve the contamination estimates, which in the present case meant multiplying the observed contamination rates by about 9. This meant that the estimates and their standard errors were rather high.

The origin of migrating pollen may vary between years, and even between days, during the flowering period of the seed orchard and, according to Paule et al. (1993), there is no feasible way to obtain reliable estimates of the gene frequencies of the immigrating pollen cloud and some uncertainty has to be accepted. However, pollen of local origin must have been involved and therefore the embryo gene frequencies from a local population were used to describe the unknown alien pollen cloud. If there was long-distance pollen migration, the genetic composition of the pollen would be slightly different from that detected in the local population (Paule et al. 1993), and may have a slight effect on the estimates of the contamination rate. Furthermore, mislabelled grafts not belonging to the Heinämäki seed orchard clones could have affected these estimates, causing a slight overestimate in the pollen contamination rates.

The spacing of the grafts in the seed orchard may also have influenced the rate of pollen contamination. There was less pollen contamination in the thinned than in the unthinned parts of the orchards in 1989 and 1993, but not in 1992. However, according to Nikkanen (1993), in 1992 the pollen from grafts in the thinned parts of the orchard started to be shed earlier than in the dense, unthinned parts. One explanation for the earlier pollen shedding in the thinned parts could be a greater amount of direct sunshine and better ventilation. The time difference in pollen shedding may have been even greater in 1989 and 1993, when differences were also found in the pollen contamination rate between the thinned and unthinned areas. Some differences in the weather conditions were also evident. In 1992 the sky was clear all day during the flowering period, while in 1993 and especially in 1989 there were some cloudy and rainy days. The average daily temperature was also higher in 1992 than in the other years. In addition, flowering took place 10–14 days later in 1992 than in 1989 or 1993 (Nikkanen unpubl.).

There are large clonal differences in the seed production capacity of Norway spruce, leading to an uneven representation of families in commercial seed lots (Ruotsalainen & Nikkanen 1989). However, equal numbers of seeds were sampled from each clone for the analyses. The proportion of background pollination may vary between families as a result of clonal differences in the abundance and phenology of flowering (Nikkanen & Ruotsalainen 2000, Nikkanen unpubl.), the amount of contamination in a seed lot subsequently being dependent on the relative proportions of individual families.

High outcrossing levels were expected as a result of abundant pollen contamination. The estimated outcrossing rates in the Heinämäki seed orchard were also high in all years and in all parts of the orchard, even though the ramets were planted in a clonal-row design, with a distance between ramets of 6.5 m. El-Kassaby (1997), however, showed a lower outcrossing rate in a clonal-row *Tsuga heterophylla* [(Raf.) Sarg.] seed orchard than in a randomly designed orchard, but did not find any between-orchard differences in seed production. In this study, the outcrossing and contamination estimates were calculated for germinated seeds, and the germinability of the seeds was fairly low. It is possible that the real degree of outcrossing in the seed orchard seed was lower, but the inbred seeds did not germinate. If this is the case then inbreeding had decreased seed germinability and seed crop production, and had also affected the proportion of pollen contamination.

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VI

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

Teijo Nikkanen, Anne Pakkanen & Jaakko Heinonen

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 in 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 seed 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 shedding is efficient (ADAMS 1992). Effective pollen distribution is especially responsible for gene flow (KOSKI 1970) and, because of the strong gene flow; conifer populations have a 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

shedding 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).

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 (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 micro-satellites (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. The data collected during several years in the seed orchard, which was the object of the present study, have earlier been used to determine the variation in flowering abundance (NIKKANEN & RUOTSALAINEN 2000) and flowering phenology (NIKKANEN 2001), as well as to estimate pollen contamination (PAKKANEN *et al.* 2000) and genetic diversity (NIKKANEN & RUOTSALAINEN 2000).

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 spacing of the grafts was 3.5 x 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 (Fig. 1). For more details about the growth and flowering of the grafts, as well as management of the seed orchard, see NIKKANEN and RUOTSALAINEN (2000), and NIKKANEN (2001).

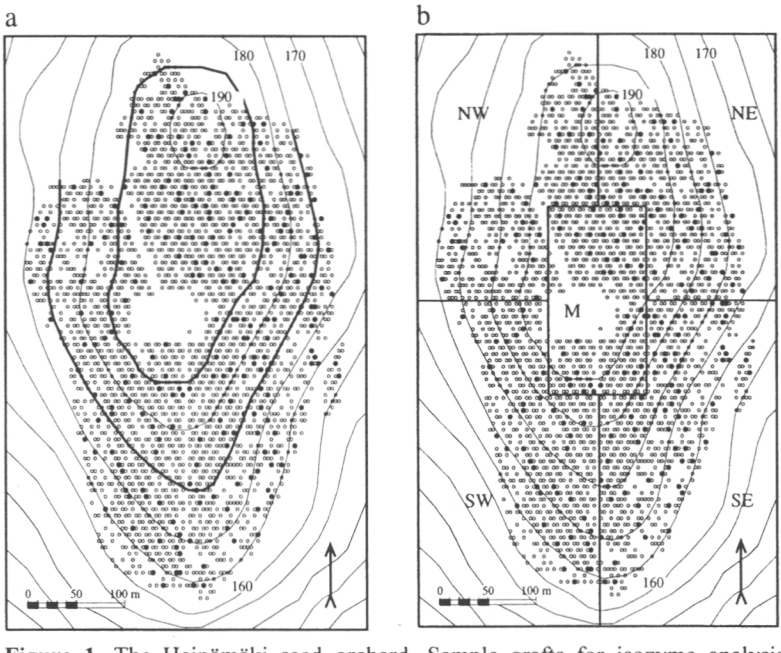

Figure 1. The Heinämäki seed orchard. Sample grafts for isozyme analysis, as well as the borderlines of a) altitude zones, and b) sections of the orchard, are marked on the map.

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. This information was utilised when the orchard was divided into zones and sections (Fig. 1).

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 north-east of the seed orchard, and from our own weather station (Datataker 610) in 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 our own weather station consisted of continuous temperature, illuminance, humidity, precipitation, and wind speed and direction during the flowering period (Table 1, Fig. 3).

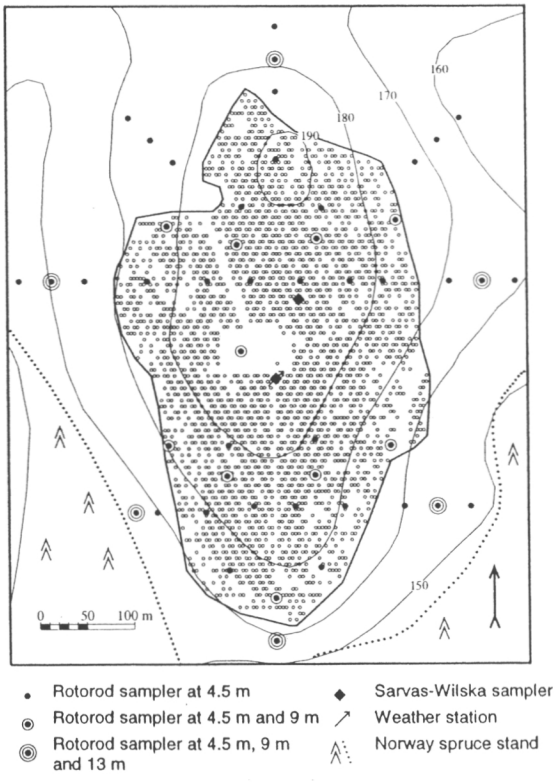

Figure 2. The Heinämäki seed orchard and its immediate surroundings. Locations of the pollen samplers and the weather station, and the borderlines of the orchard and the spruce forest, are marked on the map.

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/m²	Humidity %	Wind speed m/s	Wind 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	26.6	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

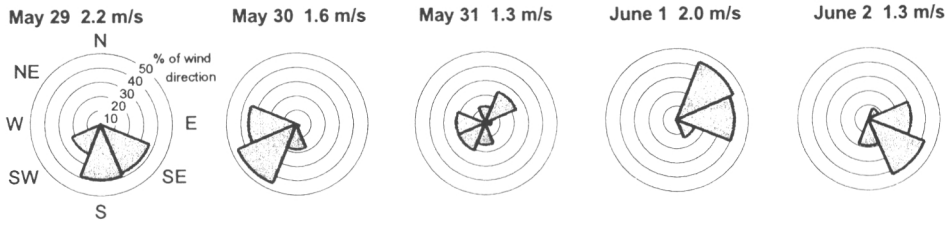

Figure 3. Distribution of wind direction in the Heinämäki seed orchard on different days during anthesis in 1995.

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). 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 were collected from the seed orchard in September 1995. A total of 490 grafts from 66 clones were sampled for the target of collection. 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 each sampling time were interpolated for the 20 x 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 the 52 clones available 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 (Table 4). 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 with 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 in 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 temperature 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 the only slight rain occurred on June 2. During the period wind direction, as well as wind speed, varied from day to day (Fig. 3). The maximum wind speed varied from 3 to 4 m/s, except on May 29 when it was 4.7 m/s. The weather conditions during the rotorod sampling are shown in Table 1.

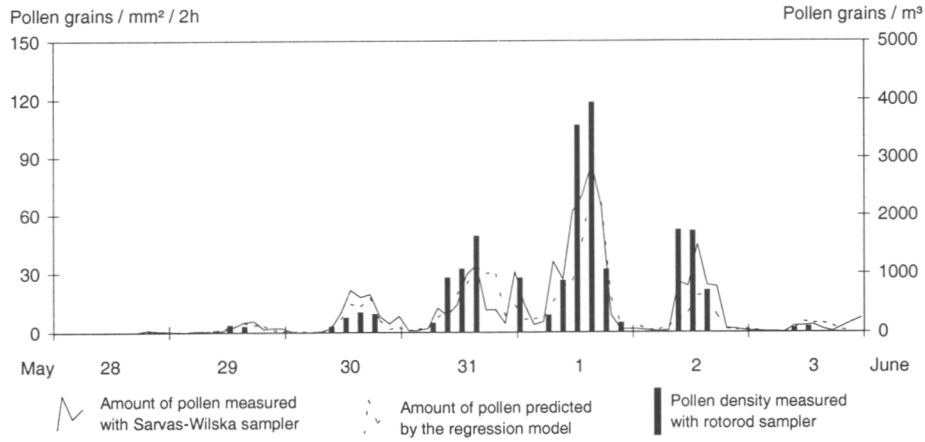

Figure 4. Variation in the amount of airborne pollen caught with different pollen samplers, and the prediction of the variation during the flowering period in 1995 in the Heinämäki seed orchard.

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 /m^3 of air (Fig. 4). 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 = 0.000). The amount of pollen measured by the two different types of pollen sampler was also highly significantly correlated (r = 0.94, p = 0.000).

Diurnal variation in the amount of airborne pollen was great; at night and in the early morning much less pollen was in the air than during daytime (Fig. 4). The logarithm of the amount of pollen was expressed as a function of temperature sum, humidity and wind speed. The estimated model was

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

where ln = natural logarithm

I_{143} = 1 if temperature sum ≤ 143 d.d., and I = 0 if temperature sum > 143 d.d

D = temperature sum at a specific moment

H = humidity at the same moment

W = wind speed at the same moment

The model is applicable when the temperature sum is between 85 d.d. and 200 d.d.. The model has two phases; the increasing phase when the temperature sum is ≤143 d.d., and the decreasing phase when it is >143 d.d.. The effects of humidity and wind speed are the same in both phases. The adjusted R^2 of the model was 0.80, and the fit of the model is shown in Fig. 4.

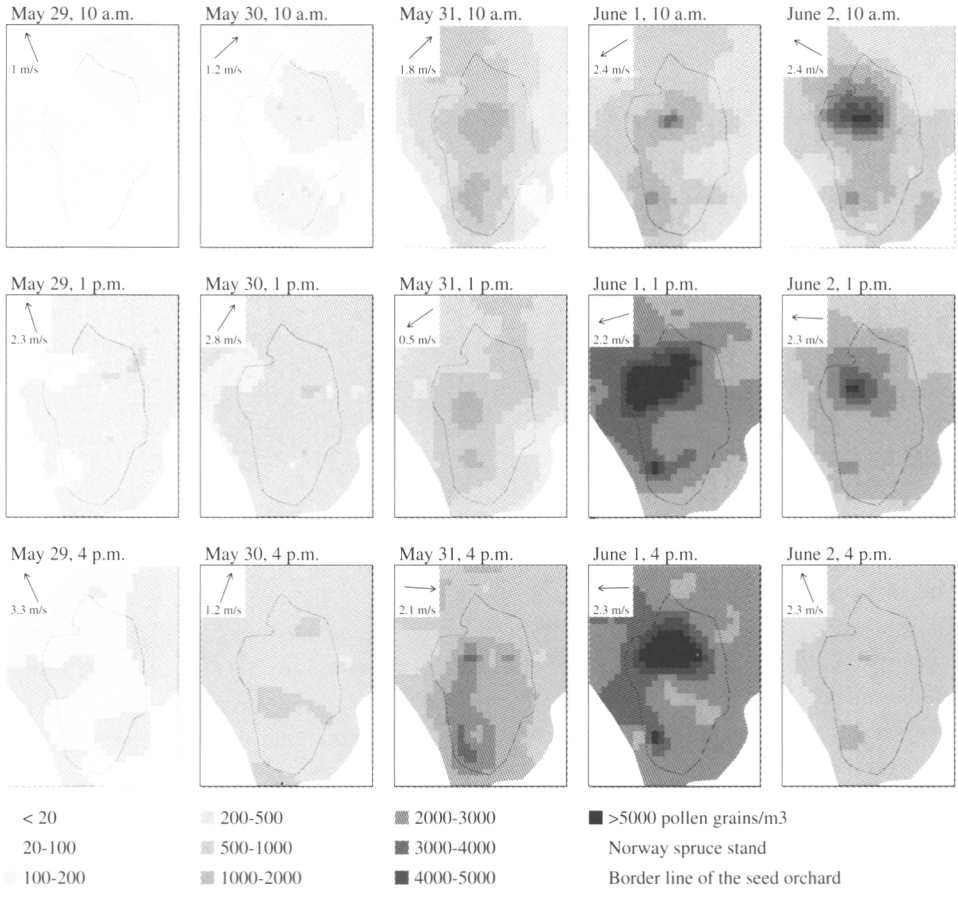

Figure 5. Spatial variation in pollen density at different sampling times in 1995. Wind speed and direction are marked on the maps.

Spatial variation in pollen density at different sampling times is shown on the maps in Fig. 5. On the first and second day of anthesis the differences in the different parts of the orchard and outside it were small, but later on they were much larger. In the afternoon of the third day the highest pollen densities were measured on the southern slope, while on the next day and the day after 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 (see Figs. 1 and 5).

On the first and second day of anthesis the average pollen densities in the seed orchard and outside it were about the same, but on the third day the average density in the orchard was more than 3 times higher, and on the fourth and fifth day about 1.5 times higher than outside it (Fig. 6).

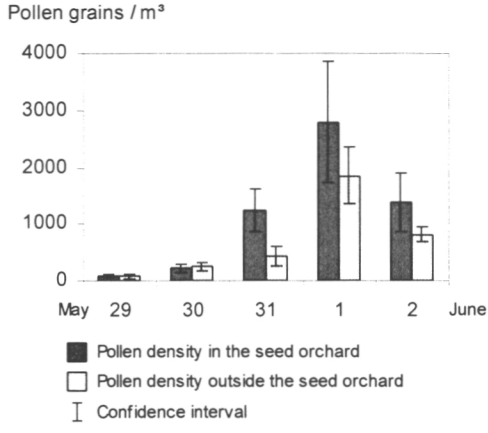

Figure 6. The average pollen densities on different days in 1995 inside and outside the Heinämäki seed orchard.

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 tested 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 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 ($r = 0.528$, $p = 0.000$).

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).

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 seeds per cone	1000-seed weight g	Percentage 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 seeds per cone	1000-seed weight g	Percentage 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

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 seeds 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)

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 (Fig. 1, Table 4). The differences in the rate of pollen contamination were significant 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.

Table 4. Number of analysed seeds, detected and estimated pollen contamination and self-fertilisation rates and their standard deviations in different altitude 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)
χ^2		6.032	174	2.675
P		0.049	0.000	0.263

b

Section of the seed orchard	Number 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)
χ^2		6.250	181	3.882
P		0.181	0.000	0.422

DISCUSSION

In the Heinämäki seed orchard flowering in 1995 occurred some days later (May 29 to June 2) than on 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 lasted only 5 days. In years with colder and more cloudy and rainy weather, the duration of flowering is longer. The average duration of anthesis in seven different years in Heinämäki has been 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 temperature sum, air humidity and wind speed to give a rather good fit for predicting the variation in the amount of pollen (Fig. 4). 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 temperature 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. 4). 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/s). 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 for instance by SARVAS (1955). He presented an example similar to ours; in a natural Scots pine stand, when the humidity is low the amount of pollen is high even at night (SARVAS 1962).

When the spatial variation in pollen density was investigated in detail (Fig. 5), 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 in the seed orchard implies the effect of wind, and phenological differences in pollen shedding between the southern slope and the northern part of the orchard (NIKKANEN 2001). On May 31 the highest densities occurred on the southern slope, but on June 1, especially in the afternoon, they occurred in the northern part of the orchard. On this day high densities were observed in the western, downwind side of the orchard, indicating heavy pollen shedding from the grafts and 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 (Fig. 3). 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 5).

The average pollen density in the seed orchard was about the same as that outside it during the first two days of anthesis, but on the third day it was much higher in the orchard (Fig. 6). This seems to indicate that pollen caught in 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, 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). In 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. 5 and 6), most of the female flowers in the orchard had been receptive from one to two days and, on the next day, some of them had already started to close (NIKKANEN 2001). This means that part of the orchard pollen was too late to pollinate the female flowers. This also confirms that a phenomenon, called metandry (i.e. female flowers are receptive before male flowers shed pollen), which is characteristic for Norway spruce and Scots pine (SARVAS 1962, 1968), is 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 Heinämäki seed orchard was 0.06, 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 for Heinämäki in 1995 was lower than that estimated by XIE and KNOWLES (1993) 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 selfing rates estimated for Norway spruce stands have been higher than those for seed orchards (MÜLLER 1977; LUNDKVIST 1979; MUONA *et al.* 1990). The differences in the rate of selfing between the different parts of the orchard (Table 4) were not significant.

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 the only study apart from ours (as far as we know) 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, as PAULE *et al.* (1993) also stated.

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 sum between the sections occurred on the fourth day of anthesis (Fig. 5). 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 from the seed orchard seed 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 result that the greatest increase in pollen density occurred outside the seed orchard one day later than inside it (Fig. 6), 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 of grafts 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 grafts 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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