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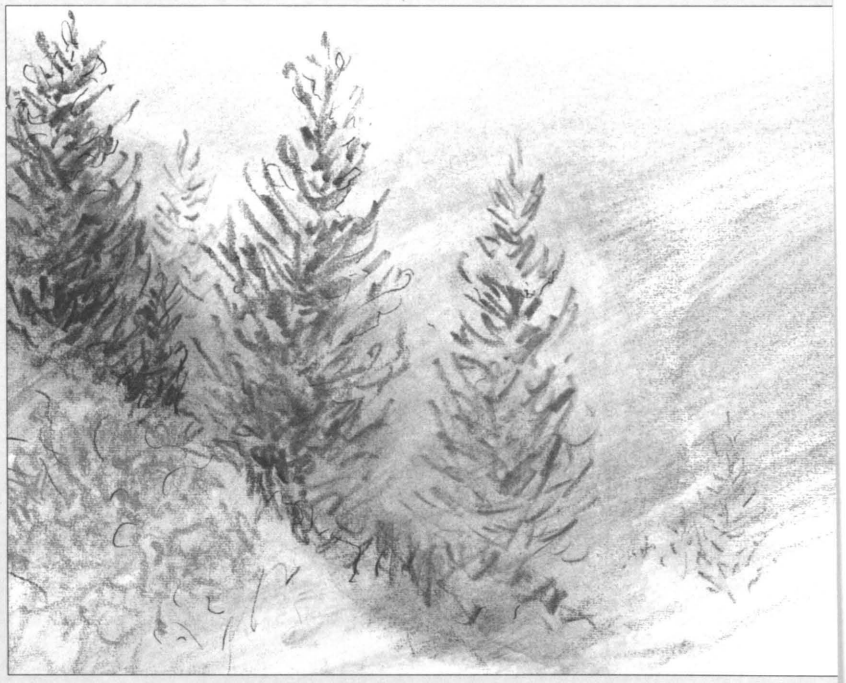

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Germinability of Norway Spruce and Scots Pine Pollen Exposed to Open Air

Katarina Lindgren and Dag Lindgren

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Germination of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) pollen decreased during exposure to open air conditions. Usually more than half of the pollen remained germinable after a few days outdoors, but following more than four days outdoors the germination became very low. This study supports the opinion that pollen in the atmosphere remains viable long enough to allow for long-distance gene flow by pollen migration, as an important factor in genetic management of conifers and in evolution, maintaining diversity and potential for adaptation.

Keywords conifer pollen, gene flow, pollen viability.
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1 Introduction

Most conifers are wind-pollinated. For many conifer species pollen grains are easily transported by the wind. Pine pollen has been found far away from its sources (Andersson 1963) and high above forest stands (Koski 1970). Mass pollen transport over a distance of about 100 km from and 2 km above the pollen-shedding stands is often supposed. Some pollen travel thousands of kilometers (Scamoni 1955). That pollen is physically able to move large distances is, however, no proof that it actually is able to carry viable genes over large distances. In order to contribute to the gene pool pollen must survive in open-air conditions during the time the transport takes place.

Eight hours exposure to sunlight through an open window during the summer of 1944 in Göttingen, sometimes reduced pollen germination of *Pinus montana* and sometimes not (Werfft 1951). Experiments with UV-irradiation indicated that this component of sunlight was the cause of a decrease in germination (Werfft 1951).

The purpose of the present investigation is to get indications of how long pollen will remain viable, by measuring germinability following exposure outdoors.

Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) are the ecologi-

cally and economically most important tree species in northern Europe. They produce large regional pollen clouds. Their patterns of gene flow by pollen are of considerable practical and ecological significance.

2 Material and Methods

Samples of Norway spruce and Scots pine pollen were exposed to open air on the roof of our office building, about 5 m above ground. The building is located at Umeå, latitude 63°50' N, longitude 20°17' E and altitude 10 masl.

Pollen that had been stored dry and deep-frozen in sealed containers was used. The day before exposure the pollen was moved to +4 °C and, a few hours before exposure, to room temperature.

To avoid severe distortion of results from the use of atypical pollen, two pine and two spruce pollen lots were used according to Table 1.

The exposures started on different days between April 29 and May 18 in 1993, before onset of local pollen shedding, when rain and strong winds were not forecast and when the sky was clear.

Ten test series with Norway spruce pollen and eleven with Scots pine pollen were performed (cf. Fig. 1 and 2). Each series comprised 5 to 8 treatment periods, including a time-zero control. We aimed for a series with exposure periods for 0 and 6 hours, 1, 2, 4 and 8 days, but there were some deviations between series. Zero hours means a few minutes of exposure. For each treatment period one exposure with pollen and one without pollen (as a control for background pollen levels) were performed.

On four separate occasions, when the weather was clear and sunny, 10 replications exposed at the same time for 24 hours were made. This was done to guarantee some exposures to high levels of natural UV-light and to get figures on reproducibility. Average daily temperatures recorded at Umeå airport at that time of exposure, on May 7, 9, 10 and 18 were 10.9, 10.6, 12.2 and 13.0 °C respectively.

Exposures were made on filter paper. For each exposure, circular filter paper (7.5 cm diameter) was fixed with paper-clip on a circular wire netting, horizontally mounted on a pole (like a mushroom) and inserted into sand in a pot (3.5 l, 18 cm in diameter). This was made in order to keep pollen on the filter paper, and to dampen vibrations in the paper (which could be caused by strong wind) and reduce risk of contamination. The wire netting and pole helped to keep pollen dry and to expose it to the sky, sun and the open air. Small amounts of pollen of both species (about 0.0021 g spruce and about 0.0036 g pine pollen) were spread as evenly as possible on each half of the same filter paper. During periods of rain, the experimental setup was temporarily covered by a plastic shelter (otherwise the pollen would clump, be washed away or germinate). Pollen was partly lost by vibration of filter paper, but usually much of the initially deposited pollen remained for the test periods.

Table 1. Pollen lots used for pollen germination test.

Species	Year of pollen harvest	Source of pollen harvest
Norway spruce	1989	Hissjö seed orchard
Norway spruce	1989	G 10 (clone SO2E1006)
Scots pine	1992	Graft archive at Sävar
Scots pine	1992	T 214 (clone SO1W4009)

Clean equipment and sand was used for each experimental series.

Samples set out at the same time were assigned in a randomized design.

After exposure, pollen was transferred to a germination medium in a Petri dish. Petri dishes containing pollen deposited on filter paper by us will be called a plate, and a "control" plate (filter paper without pollen deposited by us) will be called a blank (plate) in the following. Blanks were used to check occurrence of pollen brought by air (either from outside or between experimental plates). Blanks were not made for exposures shorter than one day.

A germination medium with 0.75 % agar and 2.5 % sucrose was used (Johnson 1943). As trace amounts of boron in the medium often stimulates germination (Stanley and Linskens 1974, p. 68–69), media without and with 0.01% boric acid were used. The average pH of the media was 6.37. The optimum agar pH range for pine pollen is between 5.5 and 6.5 (Goddard and Matthews 1981).

The Petri dishes were incubated in darkness at 28° to 30 °C, for 2 to 3 days. It was felt that the incubation time should not be too short, as germination must be given time to occur, but not too long, as pollen tubes will form a web where it becomes hard to assess if an individual pollen grain has germinated and further on, the growth medium often becomes infected by fungi.

Germination percentages were determined by examining at least 100 and usually closer to 300 pollen grains per species and plate under the microscope at 80× magnification. According to Eriksson and Jansson (1989), it is sufficient to analyse 100 pollen grains. Pollen grains with germ-tube lengths exceeding the diameter of the grain were regarded as germinated. The counts were made preferably on parts of the Petri dish where the pollen was evenly dispersed, as groups of pollen with higher concentration may have higher germination than widely spread single pollen grains (Goddard and Matthews 1981).

3 Results and Discussion

The replicated 24-hour exposures were used to study the effect of boron in the germination media, effect of source of pollen harvest and reproducibility of results. Boron did not have any significant effect on pollen germination, and therefore the differences in media will not be further considered. As the two different pollen sources of spruce and pine used showed similar effects on germination (data not shown), the results within each species have been pooled.

Germination of 30 spruce and 38 pine pollen replications (Table 2) exposed for 24 hrs, showed a standard deviation of 8.5 percent for spruce and 6.2 percent for pine (expressed in % germination units within day and species). These values, indicating the experimental error, are small compared to the variation between series. Germination of both pine and spruce pollen exposed on May 9 was low. It seems likely that there was an external reason connected to outdoor conditions, as such low values were never found after short, or zero-time exposures. But, we do not exclude that unproper thawing of the pollen exposed that day may be a contributing factor.

Table 2. Average percentage of pollen germination after 24 hours exposure, initiated at given dates (3 × 10 spruce and 3 × 10 + 8 pine replications).

Species	Date			
	May 7	May 9	May 10	May 18
Norway spruce	85	19	75	-
Scots pine	97	15	90	89

Germination of spruce pollen exposed over 5 to 8 test periods decreased with time of exposure (Fig. 1). Although the data points are scattered, it is evident that a large fraction of pollen grains on most of the plates was able to germinate after a few days and that high pollen germination was never obtained after more than four days (100 hours) exposure. More than half of the pollen grains germinated in 73 % of the plates exposed up to four days. Only one plate exposed longer than four days exceeded 23 % germination. Chi-square tests for frequency of plates with germination above 50 % revealed a highly significant difference ($p < 0.001$) between exposures shorter than four days, compared to those exposed more than four days.

Similar results were obtained for pine pollen (Fig. 2). Two-thirds of 54 pine plates exposed up to four days had germination rates higher than 50 %. No plate out of the 21 exposed longer than 4 days reached even 20 % germination. The difference between plates exposed to less than four days, compared to those exposed for longer periods, was strongly significant.

A difficulty with exposing pollen samples outdoors is that alien pollen brought by air, not initially spread on the filter paper, can be a disturbing factor. The occurrence of alien pollen before May 15 for spruce and before May 18 for pine was negligible. Later a variable amount of alien pollen occurred. Long exposures are expected to be affected more than short exposures, as the number of initially exposed pollen grains decreases over time (they blow away), while

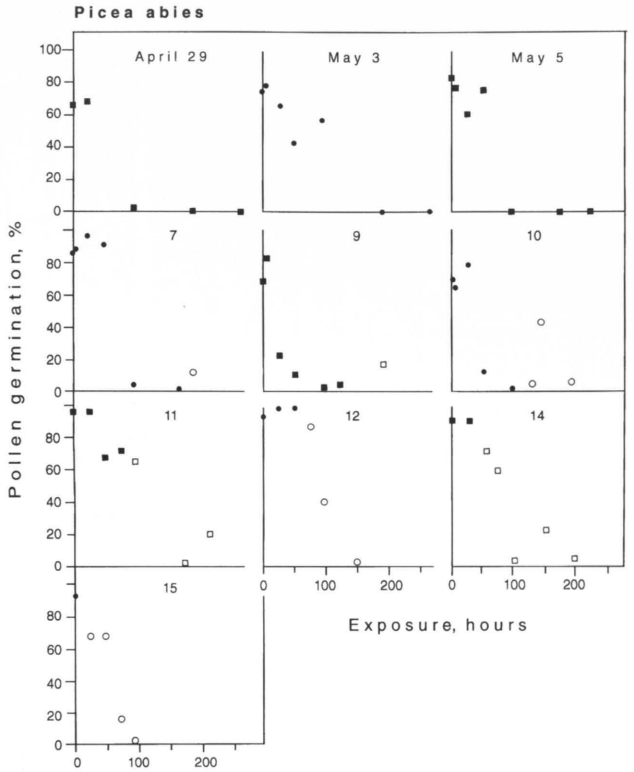

Fig. 1. Germination of Norway spruce pollen exposed to open-air conditions outdoors for 0 to 11 days (0–264 hours) for 10 test series started between April 29 and May 15. Solid symbols are used for observations made before May 15 and open symbols denote observations after May 15, which may be affected by an occurrence of disturbing foreign pollen brought by the air.

alien pollen grains accumulate. The germination of alien spruce pollen found on blanks varied between 5 and 80 percent on different days between May 15 and May 22. The corresponding values for pine pollen were 1 to 69 %. The average numbers of pollen grains per blank varied during those days between 19 and 765 for spruce and between 3 and 541 for pine. Probably, alien pollen caused an over-estimate of germination for long exposures. Values, which may be over-estimated because of alien pollen, are indicated by open symbols in Figs. 1 and 2. The conclusion that only a small fraction of pollen remains germinable more than four days seems trustworthy even when alien pollen is considered.

Table 2, Fig. 1 and Fig. 2 indicate that there was a large variation in pollen germinability depending on the day of exposure. The statistical error, as judged from the replicated 24-hour exposures (Table 2), is far too small to explain germination differences obtained after 24 to 100

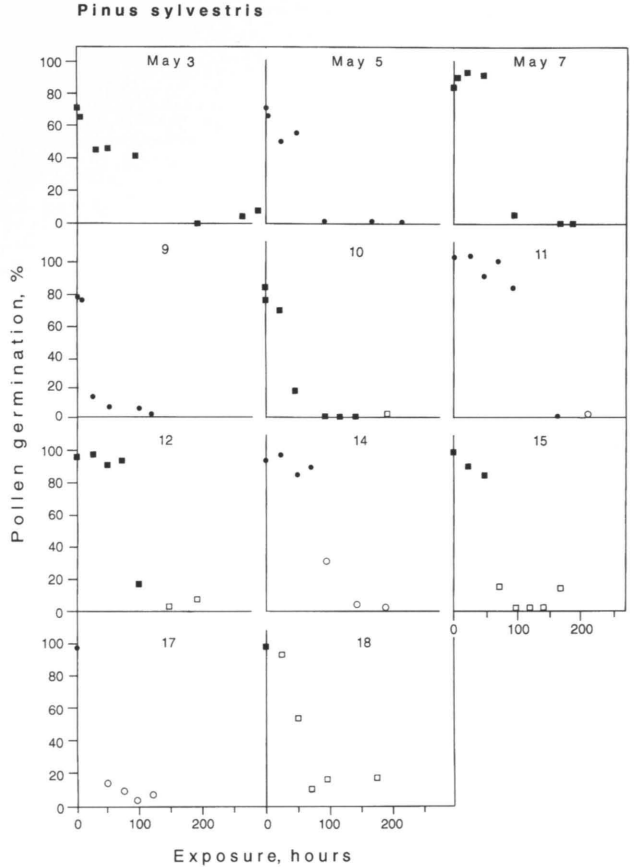

Fig. 2. Germination of Scots pine pollen exposed outdoors for 0 to 12 days (0–288 hours), obtained from 11 series started between May 3 and May 18. Records which may be affected by external pollen (May 18 and later) are denoted by open symbols.

hours exposure. Furthermore, as such large variation was not found after zero hours exposure, it seems that the cause is likely to be meteorological. Different atmospheric humidity, the temperature of the air and the different intensity of sunshine during different days seems to have influenced the germination of exposed pollen to a different extent.

The present results support the opinion that pollen may stay viable in the open- air conditions for several days, rather than for a fraction of a day. Of course, lying on filter paper is a different environment than flying free in the air. It is difficult to devise an experiment closer to the latter situation. The present study supports the hypothesis that pollen may fall to the ground during the night and be lifted again the next day by air turbulence, caused by the sun warming the ground, without losing its viability for several days. The over-night rest may be spent on a

needle or blade of grass, and the filter paper may mimic these conditions.

Classification of pollen in our test procedure as germinable or non-germinable is not equivalent to the ability to fertilize in vivo. The impression from the literature, as well as from own experience, is that the outcome of the pollen germination test is easily influenced by different environmental conditions, such as air moisture, temperature and intensity of sunshine, that may not be closely associated with pollen viability. For example, in some of our tests (data not shown), the germination was considerably lower if pollen was brought to the germination test after 6 hours exposure at room temperature indoors, than if it was exposed for 6 hours outdoors. However, similar germination tests as used in this study are widely used for prediction of fertilization ability of conifer pollen for practical purposes. Pollen germination on agar has been shown to be highly correlated with pollen germination *in vivo* (Jett et al. 1993). Determination of pollen viability by germination has been suggested as superior to other alternative methods to predict pollen viability (Ho 1992).

Experiments with controlled treatments of pollen (such as UV-irradiation or moisture regimes) can never exactly mimic real conditions to which a pollen grain *in vivo* is exposed, nor can exposure of pollen attached to the ground. Nevertheless replicated experiments, such as made here, can at least ensure that many typical circumstances are considered. Thus, experiments like this while never conclusive can be indicative. Furthermore, gene-flow is also a phenological issue, as there must be receptive female strobili at the far end of a pollen trajectory. From that point of view, some hundred kilometres are unlikely to form a barrier (Lindgren et al. 1995).

Pollen of Scots pine and Norway spruce can maintain its germinability and thus probably its viability in open-air conditions for several days. If conditions are suitable, pollen may be transported by the wind for thousands of kilometres in a few days (cf. Lindgren et al. 1995). Thus, the current investigation supports the idea that long-distance gene flow by pollen migration may be an important mechanism for adaptation, evolution and genetic structure of conifer forests. The gene flow may also be strong enough to have a considerable effect on the genetic characteristics of individual seed harvests from stands or seed orchards.

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