enhanced when they were co-pollinated with dead pollen of another elm species (Alan A. AGER, personal communication, and unpublished data from our experiments on 'mentor pollen'). Thus, in some crosse that are actually incongrous, seed set may be enhanced over self controls, which makes it appear that the hybridization worked. It is therefore essential to monitor selfing and to authenticate the hybrids (in the breeding work as is commonly done in elm) especially when trying the effectiveness of 'mentor pollen'. In some cases morphological characters are suitable, but biochemical markers may be more reliable. For this purpose the quantitative determination of elm leaf flavonoid glycosides by HPTLC followed by discriminant analysis looks promising in identifying hybrid progeny of controlled and natural cross-combinations (HEIMLER et al., 1990a, 1990b, and another work in preparation).


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# Variation of Pollen Contamination in a Scots Pine Seed Orchard


By R. YAZDANI[1]) and D. LINDGREN[2])



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### Summary

A grafted Scots pine seed orchard at latitude 62 °N at the Baltic coast was studied for the occurrence of pollination from nonorchard origin (contamination). Altogether 2318 seeds were analyzed by means of isozyme embryo



[1]) Department of Forest Genetics, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

[2]) Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden

endosperm assay. The average contamination was similar in two consecutive years, but there was a significant year x block interaction, demonstrating the presence of differences between blocks depending on the year of harvest. The contamination was highest in a windward corner of the orchard and lowest in the center, but the reduction in the center was not large. Clone effects, clone · block and clone · year interactions were not significant. The impact of nondetected contaminating pollen on the estimate of contamination was calculated.

*Key words:* Background pollen, pollen migration, *Pinus sylvestris*, allozymes, isozymes.

## Introduction

In Sweden today there are 49 seed orchards of Scots pine (*Pinus sylvestris* L.) in productive age which were established during 1950 to 1960. Seed orchards consist of grafts of selected trees from natural stands. At present, 70% of planted Scots pine originate from seed orchards.

A serious problem with seed orchard seeds is that it seems that much of the pollen appears to have an origin from outside the orchard. This has frequently been found for Scots pine in northern Scandinavia (Nagasaka and Szmidt, 1984;El-Kassaby *et al.*, 1989; Hariu and Muona, 1989, 1989 Wang *et al.*, 1991) as well as in other species and places (Friedman and Adams, 1985; Smith and Adams, 1983; El-Kassaby and Ritland, 1986; El-Kassaby *el al.*, 1987). A better description and unterstanding of the pollen-contamination problem is thus vitally important.

In this paper we have studied the degree of pollen contamination in several blocks, many clones and different year crops in a seed orchard of *Pinus sylvestris*. El-Kassaby *et al.* (1989) reported on the contamination level in the same seed orchard in one block in a single year, as well as outcrossing, fixation indices and allelic frequencies. The present study is based on six times more seeds and the objectives are different.

## Materials and Methods

*Orchard description*

Material for this study was obtained from one Scots pine seed orchard (T 406 Bogrundet: lat. 62°30', long. 17°30' and alt. 5 m). The seed orchard is located at the major nursery Bogrundet, north of Sundsvall close to the Baltic sea.

The age of the grafts since planting was 17 to 18 years at cone collection. Commercial seed harvests were made in the seed orchard in 1985 and 1986 (7.1 kg seeds per hectare). However, the male flowering has increased considerably since the investigation was made. Male strobili have observed since 1980. Objective quantitative estimates of pollen production were made in 1986 (6.6 kg/ha), 1987 (5.5 kg/ha) and 1988 (30 kg/ha). Subjective records indicate that the pollen crops in 1984 and 1985 were similar to those in 1986 and 1987 (Ola Rosvall and Torbjörn Lestander, pers. comm.). Thus the studied object may be described as an orchard which has reached almost full seed production, which is harvested and used in forestry on a large scale, but which has not yet reached a high level of pollen production, and can be predicted to be less affected by contamination today than when this study was made.

The orchard occupies an area of 12.5 hectares. There are several hundred meters from the orchard fringe to the closest pine tree. The trees were spaced 5 m apart within rows. Rows are separated by 7 m. Tree height was approximately 5 m. The clonal origin was from the interior of northern Sweden, the average latitude was 65.8° and the average altitude 440 m. a. s. l.

*Seed samples*

In September of 1985 and 1986, open pollinated cones were harvested from 40 clones. The samples were drawn form one ramet per clone in three different areas of the orchard (A, B and C) along the previailing wind direction during pollen dispersal. The areas will be called block. They are situated in the assumed windward and leeward corners of the orchard as well as in the middle. Cones were collectet at breast height from the southern aspect of each tree, and were dried at room temperature. Seeds were extracted, cleaned and stored at —20 °C until analysis. Altogether 2318 seeds were analyzed using isozyme embryo endosperm assay.

*Isozyme analysis*

Twenty-one loci were used to estimate the pollen contamination levels. The following systems were used (number of loci scored in brackets if more than one): Acid phosphatase, Aconitase, Alcohol dehydrogenase (2), Fluorescent esterase, Glutamate dehydrogenase, Glutamate oxaloacetic transminase (2), Leucine aminopeptidase (2), Malate dehydrogenase (2), Menadione reductase, Phosphoglucomutase (2), Phosphoglucose isomerase (2), 6-Phosphogluconate dehydrogenase (2) and Shikimate dehydrogenase (2). For references on the technique see El-Kassaby et al. (1989).

*Evaluation of contamination*

The multilocus genotype of pollen gametes originating from open-pollination was deduced from the comparison between allozyme patterns in each megagametophyte and its corresponding embryo. Then the multilocus genotype of pollen gametes was compared to all possible combinations of gametic genotypes that can be produced by the orchard clones. The frequency of these pollen gametes that did not match any of the within-orchard combinations was used to estimate the contamination level. It is important to note that any pollen gametes from outside that carry a genotype similar to any of those within the orchard will not be detected. Therefore, the contamination level estimated may be considered a "minimum" estimate.

A simulation of what the pollen genotypes would have looked like if all pollen originated from outside the orchard was made by replacing the experimentally determined alleles with a set of randomly derived alleles based on the assumed allele frequencies in the contaminating pollen population (cf. Wang et al., 1991). The gene frequency assumed for the contaminating pollen population was set to the average of the gene frequencies of seven Swedish Scots pine seed orchards (unpublished results). These clones cover the whole range of Scots pine in Sweden. No effect of linkage was assumed in the contaminating pollen. This simulated data set was analyzed in the same way as the experimental data. As the simulation was mage only once, it is subject to sampling error. The main reason for a simulation approach instead of exact calculations was the presence of missing data at some loci. Exact calculations are different for each multilocus constellation of missing data.

We believe mistakes are sometimes made in the long series of steps preceding the identification of a contamination (Szmidt, 1991, in prep.). Mistakes are likely to cause

overestimates of the contamination level. A systematic error in contamination is likely to be approximately the same for different years, blocks and clones. Thus, the conclusions on variation in contamination level will be reliable, even if the absolute level is uncertain.

*Analysis of variance*

An analysis of variance of the outcrossing percentage was performed using the GLM procedure of the SAS program package, using the following model:
$Y_{ijk} = \mu + Y_i + B_j + C_k + Y^*B_{ij} + Y^*C_{ik} + B^*C_{jk} + e_{ijk}$
Year (Y) and Block (B) were considered to be fixed effects, while clone (C) was considered random.

The observed mean squares are the same regardless wether the factors are considered to be fixed or random, but the difference is important for the equations of estimated mean squares and the construction of F-ratios. The blocks were chosen systematically because of their location, and do not constitute a sample of anything, thus blocks should be considered as fixed effects. Two consecutive years can not be considered as a random sample of years as the climate between edjacent years is correlated and as a seed orchard has a development over time, e. g. pollen production increases. Thus it seems best to consider years as a fixed effect. For clones, it is natural to regard them as a sample of all possible clones, and thus it seems appropriate to consider them as random effects.

The individual observations consist of the percentage proven contamination for a clone in a block, based on 10 seeds (a few cases with 9). The variation in observed values was not large enough to justify a transformation. Only data from 34 clones in which values for all six combinations had been obtained were used to keep the data balanced.

*Wind direction*

The frequency of wind directions in June at Härnösand (30 km north of the orchard site) during 1931 to 1960 was used (Taesler, 1972). As the frequencies are similar to those of Umeå and both these sites are approximately at the same distance from the coast as the seed orchard, we assume that they are representative.

## Results

The analysis of variance including expected mean squares and appropriate F-tests, is shown in *table 1.*

The variance ratios and the corresponding probabilities presented are based on a model regarding clones, blocks and years as fixed effects. There was no indication of any differences between clones or years, and neither of any interaction between clone and block. The interaction between clone and year was smaller than expected for statistical reasons alone, but this can be accepted as statistical noise. There was a difference between blocks and a significant interaction between blocks and years. Thus, it is concluded that the contamination is different in different parts of the seed orchard, and that these differences change between years.

*Table 1.* — Three-way analysis of variance (clones, years and blocks) of percentage "minimum" contamination.

| Source | DF | SS | MS | F-value |
|---|---|---|---|---|
| Clone | 33 | 17157 | 520 | 1.04 |
| Year | 1 | 253 | 253 | 0.88 |
| Block | 2 | 3119 | 1560 | 3.29* |
| Clone x Block | 66 | 31313 | 474 | 0.95 |
| Year x Block | 2 | 3748 | 1874 | 3.76* |
| Clone x Year | 33 | 9500 | 288 | 0.58 |
| Error | 66 | 32877 | 498 | |

Comments and explantations: DF = Degrees of Freedom; SS = Sum of squares. MS = Mean square = SS/DF.

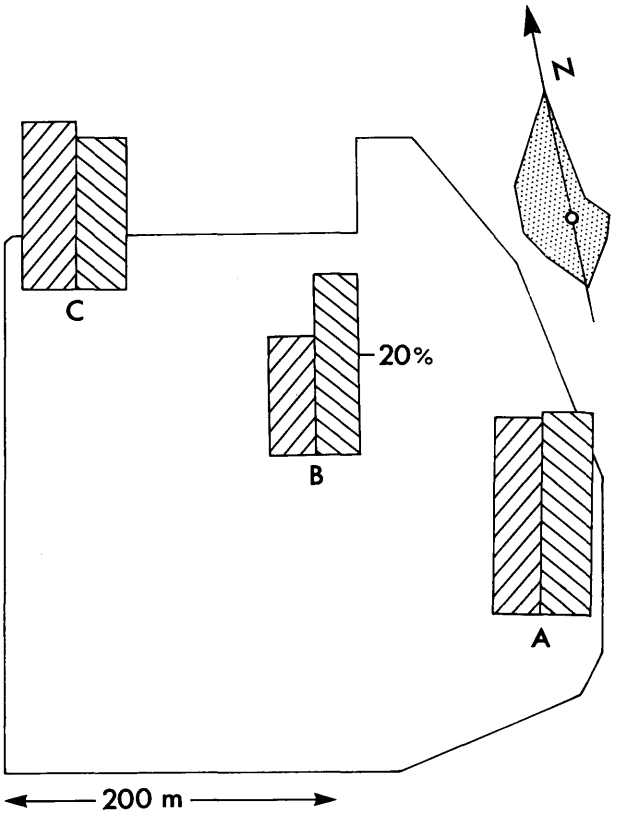


*Figure 1.* — Configuration of Bogrundet Scots pine seed orchard. "minimum" contamination levels are demonstrated in three different parts of the orchard (A, B and C). Frequency of different winddirections (north means wind blowing from the south to the north) is indicated.

The average contamination observed is 35% and thus the expected mean square caused by the binomial distribution among 10 seeds is $35 \cdot 65/10 = 228$. The observed mean square is 498. The difference is considerable. There is an additional important source of variance between observations besides clones, years, blocks and binomial variation.

The actual clonal mean values show a variation. The lowest "minimum" estimate of contamination, 13%, appeared in clones BD-2129 and AC-1093. The highest contamination level appeared in clones AC-1085 with 57% and AC 4238 with 54%. Differences between clones are, however, just as large as is expected statistically, in spite of each clone value being based on 60 seeds. It appears that some clones show both low and high contamination in different blocks and different years in a pattern we are unable to interpret.

The "minimum" contamination levels are shown in *figure 1* and the contamination levels corrected for undetected contaminations is shown in *table 2.* Many of the simulated non-orchard gametes fitted to an orchard clone, and thus the correction for nondetected contamination is large.

*Table 2.* — Proportion of embryos fertilized by contaminating pollen in different blocks and the annual seed crops of 1985 and 1986 after correction for undetected contaminating pollen.

| | Harvest year | |
|---|---|---|
| Block | 1985 | 1986 |
| A | 72 | 73 |
| B | 59 | 70 |
| C | 77 | 78 |
| Mean | 69 | 74 |

The contamination level is lower in the central part of the seed orchard than in the corners (*Figure 1*). The assumed leeward corner is less affected by contamination than the windward corner.

## Discussion

The contamination varies from year to year in some seed orchards depending on their own pollen production compared to surrounding stands (FRIEDMANN and ADAMS, 1985; HARJU and MUONA, 1989). The present results indicate that differences are not very large.

The "minimum" estimate of the proportion of the background pollination for many Scandinavian seed orchards is around 21% to 41% (YAZDANI and RUDIN, 1988). In the present study ,the "minimum" estimate of pollen contamination varied from 24% to 40%, with no differences between clones and years. In northern Sweden, seed orchards of *Pinus sylvestris* are usually transferred southward with regard to the clonal origin to improve seed maturation. Survival is a critical factor for Scots pine and southern provenances do not survive satisfactorily. Serious effects on survival may be expected because of contamination (LESTANDER and LINDGREN, 1985). Knowing the proportion and origin of the contaminating pollen, the expected hardiness of the seed orchard crop can be predicted and the crop can be used in appropriate sites. In the current seed orchard many seeds were produced while the pollen production was still low, so high levels of contamination could be predicted. If seeds produced by this seed orchard are regarded as "forbidden fruit", it implies rejecting a lot of physiologically very sound seeds. A better strategy may be to use the orchard hybrids produced in young orchards on more southern sites than those produced in seed orchards which have reached full pollen production.

In Douglas-fir seed orchards, the rate of contamination by pollen from outside sources is found to be higher for early and late flowering clones (EL-KASSABY and RITLAND, 1986). Any such effect was too small to appear as a clone difference in this experiment. Neither did HARJU and MUONA (1989) find any evident clone differences. However, there is a considerable variation in phenology within clones (JONSSON et al., 1976). In another investigation we found differences in contamination between seeds originating from early and late female strobili (YAZDANI and LINDGREN, in prep.).

One method of counteracting pollen contamination is to establish very large seed orchards, whereby large amounts of orchard pollen are produced and a small proportion of the orchard is close to the fringe. This investigation support such practices, but the improvement that can be achieved may not be very large.

It is of great importance to investigate the causes of the high proportion of pollen contamination in seed orchards, in order to find a solution based on a deeper knowledge of the problem.

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