# Effects of fluorides and sulphur dioxide on pollen germination and growth of the pollen tube

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#### Abstract

The action of fluorides and sulphur dioxides from emissions from a phosphate fertilizer factory significantly reduced the germination of Scots pine pollen grains. The pollen tube length indicated that its growth is uninhibited even though the pollen was collected under conditions of air-pollution. There are statistically significant differences showing longer tubes in the sensitive trees pollen grains. The ageing of pollen from the sensitive trees occurs probably more rapidly than in the tolerant trees.

Key words: Scots pine, fluorides, pollen germination

### INTRODUCTION

Fluorides and sulphur dioxide emitted by a phosphate fertilizer factory change the genetic structure of a Scots pine (*Pinus silvestris* L.) stand. Trees of the genotype APH-B5B5, more tolerant to these gases, are usually represented in the basic stand and in the  $F_1$  progeny, while the most readily dying, sensitive trees and their progenies are primarily of genotype APH-B6B6 as identified with the help of an isoenzyme analysis of acid phosphatase (APH) in macrogametophytes (Mejnartowicz 1983).

Trees in the emission zone have substantially injured leaves, produce fewer cones, and the majority of their seeds are empty. One of the causes of seed infertility appears to be injury to pollen caused by air-pollution.

In the study reported below the question was investigated whether air-pollution with fluoride and the accompanying sulfur and nitrogen oxides emitted from a factory of phosphate fertilizers influences pollen germination and pollen tube growth in Scots pines growing in an area affected by these emissions. Furthermore, it was investigated there are any differences in this respect between pine trees differing in tolerance to these gases.

## **METHODS**

Pollen collected in 1982 from 4 tolerant and 4 sensitive trees (1982) on the day following maximum pollen shed for Scots pine was left for about one day in the zone of fluoride pollution at a concentration of 0.012-0.088 mg·m<sup>-3</sup>·30 min<sup>-1</sup> emitted from the above-mentioned factory. From each tree the pollen from 10 strobili was shed onto Petri dishes containing 1% agar with 0.001% boric acid (Chira 1967). The dishes were kept at 30 C in darkness of 6 days. After that time the length of 20 pollen tubes in every Petri dish was measured and the percentage of germinated pollen grains calculated.

In 1983, the germination of pollen grains and the elongation of the pollen tubes was measured on pollen collected from strobili on shoots detached 3-4 days before pollination, and placed in rooms that were free of polluted air. Part of the collected pollen was sown directly onto an agar medium and part of the pollen was left for 6 days in open air.

# RESULTS

Pollen which had been collected from strobili remaining under the influence of industrial emissions (1982 experiment) germinated at an average rate of 50%, with a lower germination percentage (41%) found in the more tolerant trees than in the sensitive ones, where it was 59%. However, these differences proved not to be significant when the values were subjected to angular transformation and verified with a t-test for small samples (Table 1).

Table 1

The mean length of the pollen tube and the percentage germination of pollen grains from sensitive and tolerant trees. Differences estimated by the t-test for small samples

Tress	Pollen tube length, µm	% of germination
Tolerant	236	41
Sensitive	259	59
t-test, between groups	13.3**	NS

<sup>\*\* —</sup> Statistically significant (p>0.99).

NS — Statistically non-significant (p<0.95).

The pollen tube length was on the average 236  $\mu m$  for tolerant trees and 259  $\mu m$  for the sensitive ones. The means differ significantly at the 99% level of probability for the two groups of trees.

Pollen from both groups of trees, obtained under conditions of non-polluted air germinated very well (Table 2). The pollen tubes were very similar

in length to those in the first experiment, and the difference betwen sensitive and tolerant trees was also signficant. The pollen tube was 220  $\mu m$  long for the tolerant trees and 273  $\mu m$  long for the sensitive ones (Table 2).

Table 2

Length of the pollen tube and percentage germination of pollen obtained under conditions of clean air

Tress	Pollen tube length, µm	% of germination
Tolerant	220	98
Sensitive	273	97
t-test for small samples	4.95**	NS

<sup>\*\* —</sup> Statistically significant (p > 0.99).

The pollen collected in clean air had been left for 6 days in an open dish and exposed to the action of all atmospheric influences being protected only from rain. It had a much higher germination percentage than the pollen collected from strobili that had shed pollen in the region of pollution, and there were no statistically significant differences between the groups of trees, neither in the germination percentage nor in the length of the pollen tube, which was much shorter than tubes of pollen shed on the agar medium imediately after collection (Table 3).

Table 3

Percentage germination of pollen grains and the length of pollen tubes of pollen collected under conditions of non-polluted air and sown 6 days after collection

Tress	Pollen tube length, μm	% of germination
Tolerant	78	75 dieve al
Sensitive	ememin 72 and made	1 Latin 2 80 2 Legis (
t-test for small samples	NS	NS

NS - Statistically non-significant (p < 0.95).

#### DISCUSSION

The results presented indicate that the action of fluorides, sulphur and nitrogen oxides occuring in the emissions have significantly decreased to 50% the germination of Scots pine pollen grains, and have reduced it even more in tolerant trees than in sensitive ones. However, even poorly germinating pollen can yield good seed formation. Callaham and Duffield

NS - Statistically non-significant (p < 0.95).

(1961) investigated seed set in *Pinus jeffrey* pollinated with lots containing 100, 50, 20, 10 and 2% viable pollen. They obtained maximum sound seeds with 50% viable pollen. Unfortunately data are not available concerning the flowering phenology of pines growing in the pollution zone. We know only that there is some individual variation of the order of one day in the moment of onest of pollination in the studied pine trees. From literature, however, it is known that the earlier pollinating trees may be the main donors of male gametes (Sarvas 1962).

The measurements of the length of the pollen tube after six days of growth in vitro indicate that its growth is uninhibited even though the pollen was collected under conditions of air pollution. It is not known whether there is any biological significance in differences showing longer pollen tubes in the group of sensitive trees than in resistant ones, since in the studied Scots pine trees the minimal length of the pollen tube,  $174 \, \mu m$ , substantially exceeds the  $100 \, \mu m$  length needed for fertilization (A. Hejnowicz, personal information).

Under conditions of free access of air to the pollen, after six days the pollen tubes were already three times shorter than in the pollen sown directly after being shed from strobili, the "ageing" of pollen from sensitive trees occured more rapidly than in tolerant trees.

The pollution of air with fluorides, oxides of sulphur and nitrogen emitted by the phosphate fertilizer factory causes a reduction to about 50% of germinating pollen grains in tolerant and in sensitive trees. The length of the pollen tubes is the same in the pollen collected from strobili maturing in the pollution zone and from those which matured in pure air.

The length of pollen tubes grown in vitro was greater for the sensitive trees than for the tolerant ones.

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Wpływ fluorków i dwutlenku siarki na kiełkowanie pyłku i wzrost łagiewki pyłkowej

# Streszczenie

W populacji sosny zwyczajnej, która rośnie pod wpływem fluorków i dwutlenku siarki, emitowanych przez zakłady nawozów fosforowych, stwierdzono zmniejszenie się o 50% liczby ziarn pyłku zdolnych do kiełkowania. Zanieczyszczenia powietrza działały hamująco w podobnym zakresie na pyłek z drzew tolerancyjnych jak i wrażliwych. Pyłek z drzew wrażliwych miał dłuższe łagiewki niż pyłek z drzew tolerancyjnych i szybciej ulegał starzeniu.