

## THE EFFECT OF AIR POLLUTION ON THE ULTRASTRUCTURE OF THE DEVELOPING AND CURRENT YEAR NEEDLES OF NORWAY SPRUCE

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### S U M M A R Y

The ultrastructure of mesophyll cells of developing and current year green spruce needles was studied in the environments of two factories in Finland. The one is a fertilizer factory, emitting mainly SO<sub>2</sub> and fluorides. The other is a pulp mill emitting SO<sub>2</sub>.

Three types of injuries were found. The swelling of thylakoids seen mainly in S- and F-polluted area is thought to be caused, partly at least, by fluorides. The reduction of grana seen in both polluted areas and the lightening of plastoglobuli with appearance of lipid-like material in cytoplasm, seen mainly in S-polluted area, are thought to be caused by sulphur.

The trees studied were in moderate condition and the needles taken for study were always green. The method and material are thought to be suitable for diagnostic purposes in controlling air pollutants.

### I N T R O D U C T I O N

The long-term action of low concentrations of combined pollutants on plants is thought to be the most usual and significant situation in the field. However, structural studies concerning the effects of pollutants on plants, have mainly concentrated on the effects of a single pollutant in laboratory conditions (e.g., Malhotra 1976). The observations of combined effects have strongly relied on biochemical or physiological studies (e.g., Tingey et al. 1973). Some results at the ultrastructural level have nowadays been reported under field conditions (Godzik and Knabe 1973, Soikkeli and Tuovinen 1979, Soikkeli, in press).

This study deals with the effects of air pollutants on the developing and current year spruce needles under field conditions. The questions we seek to answer are e.g., 1) when can the first injuries be resolved in the developing green spruce needles, 2) are the changes different under the influence of different pollutant combinations, 3) are the changes similar to those

observed earlier in older needles (Soikkeli and Tuovinen 1979), and 4) which are the possibilities to use the ultrastructural methods and the observations on the developing and young needles for diagnostic purposes? The study is part of a project dealing with the effects of air pollutants on boreal coniferous forests in Finland.

## M A T E R I A L S   A N D   M E T H O D S

The material for the study was collected from the vicinity (under 500 m from the factories) of two factories located 25 km and 7 km north of Kuopio (62° 54' N, 27° 40' E) in Finland from May to December 1977. The one is a fertilizer factory, the main pollutants of which are SO<sub>2</sub>, SO<sub>3</sub>, NO<sub>2</sub>, NH<sub>3</sub>, and fluorides. In 1977 the emissions of SO<sub>2</sub> were about 2047 t/y and that of fluorides 3.5 t/y. The other factory is a pulp mill emitting mainly SO<sub>2</sub> (ca. 4000 t/y) but some ammonium sulphite and ammonia are emitted, too. The control samples were taken from stands of Norway spruce in an area, from which the structure of older needles (1-2 years) has been reported earlier (Soikkeli 1978).

The developing and current year green needles were taken from branches facing the factories, at a height of 4-7 meters. At the control sites samples were taken from the same side of trees (i.e. southwest) as in the industrial areas. The samples were taken both from the tip and middle parts of the needle, except in the beginning of May, when the developing minute needles were fixed as a whole. The methods used in transporting the needles to the laboratory as well as fixing, embedding, staining, and analysing the material have been described earlier by Soikkeli (1978).

## R E S U L T S

### Normal structure

In early May, when the needles were in buds, the mesophyll cells had a lot of cytoplasm and only little vacuole. The proplastids and amyloplasts with big starch grains were numerous and distributed evenly throughout the cytoplasm. The shape and appearance of the proplastids varied. The thylakoids contained some stroma lamellae and only scarce minute grana. Plastoglobuli were also rare; approximately two per proplastid. By the time the needles broke through the buds in the end of May, the amyloplasts were scanty and were not found later. The developing chloroplasts were moving towards the cell walls.

Only two weeks after needle breakage the grana began to develop having then 3-5 lamellae/granum. Later in summer there were appr. 5-8 lamellae/granum. The amount of plastoglobuli was also increasing from 2-3 in May to appr. 10/chloroplast in summer and winter. Tannin was observed even in the first May samples and its amount increased during maturation. Usually the tannin was as granules in the central vacuole. From July the overall appearance of chloroplasts and

the whole mature mesophyll cells and the changes in autumn and winter were similar to those reported in older spruce needles by Soikkeli (1978).

#### Structural changes near the pollutant sources

The first changes i.e. the slight swelling of stroma lamellae, in the current year needles were distinguished in August in both industrial areas (Fig. 1). Later in autumn (from September to December) the swelling spread also to the grana thylakoids in green needles collected from the vicinity of the fertilizer factory. Instead, near the pulp mill, the needles showed swollen thylakoids only in August.

The number of grana lamellae was greater in needles in the area of the fertilizer factory than in control needles in August. From that time the number of grana lamellae decreased so that in December the grana of needles collected from this area had about as many lamellae/granum as the control needles. In December the samples collected from the fertilizer factory had cells, where the chloroplasts were near the cell walls, when they normally at this time of the year are clumped together in some corner of the cell.

The green needles collected near the pulp mill showed injury with lightened plastoglobuli in August (Fig. 2). Simultaneously small lipid-like droplets appeared in the cytoplasm close to the chloroplasts. At a later stage of the injury the shape of plastoglobuli began to change and their number increased. Near the pulp mill the lamellae of grana stacks were slightly decreased so that grana had approximately 5-6 lamellae/granum.

In the vicinity of both factories the number of plastoglobuli increased more than in control needles being at least 20/chloroplast. Also the changes in the structure of tannin in the needles were similar in both study areas. Tannin was as a thick ribbon and/or as big lumps in the central vacuole of cells which then often showed changes in the cytoplasmic organelles, as well. In both factory areas the healthy looking cells in needles were dominating in the current year needles.

#### D I S C U S S I O N

The structural changes in needles from industrial areas were seen from August, when they were small. This could partly be due to the low production capacity and corresponding low emissions of factories during the summer months. Grill and Härtel (1972) observed that young spruce needles were more sensitive than the older spring ones. On the other hand, young actively growing tissue is reported to be more resistant to pollutants than older tissues of pine needles (Malhotra 1976). Earlier studies in Finland have shown that the first damage of conifers mainly takes place in older needles during winter and spring

under chronic pollution in the field (Havas 1971, Soikkeli and Tuovinen 1979). This study shows that the youngest needles reveal certain injuries after a few months' exposure to pollutants. Thus, under chronic exposure it is difficult to decide which are the more sensitive (young or older needles) because the effects slowly accumulates.

Like in previous studies made on older needles in Finland (Soikkeli and Tuovinen 1979) the different pollutants caused different types of injuries also in current year needles. The differences in the younger needles, however, were not as apparent as in older needles. The swelling of thylakoids predominates in needles growing in an area polluted with SO<sub>2</sub> and fluorides. It has been thought that this kind of swelling observed also earlier in second year green spruce and pine needles (Soikkeli and Tuovinen 1979, Soikkeli, in press) is caused by fluorides. On the other hand, the swelling of thylakoids has been often reported in material polluted by SO<sub>2</sub> (Wellburn et al. 1972). The curling of the thylakoids which was seen in older polluted needles (Soikkeli and Tuovinen 1979) was not found in this study. It is likely as reported earlier that this injury appears during winter and is caused by the pollutant (fluorides) and frost together.

Near the fertilizer plant some needles had cells where the wintering had not succeeded; the chloroplasts were along the cell walls as in summer. The weakening of the winter hardiness of spruce in the same area has been reported in the physiological studies (Huttunen et al. personal communication). The nitrogen compounds are known to retard and postpone the development of frost hardiness (e.g., Havas 1971). On the other hand, the greater number of grana lamellae compared to control needles could be due to the fertilizer effect of the factory. The reduction of these high grana stacks like the reduction obtained near the pulp mill could be, according to present and earlier studies, caused by sulphur compounds.

The changes in the amount and shape of plastoglobuli observed in current year needles have earlier been found also in older needles from the same and different polluted areas (Soikkeli and Tuovinen 1979, Soikkeli, in press). The appearance of this injury, like that of a reduction injury, in S-polluted areas indicates that S-compounds are the causal agents. According to this study the lightening of plastoglobuli develops faster than the reduction of grana lamellae, which was just beginning during the study period. In older needles from S-polluted areas the grana have only 2-3 lamellae/granum (Soikkeli and Tuovinen 1979).

One of the questions arising from the ultrastructural studies in relation to air pollution in Finland is, whether this method and material are suitable for diagnostic purposes. The earlier results from second-year green needles have been promising. This study shows that also current year needles could be used for diagnostic purposes, although all the changes and differences seen in older needles could not be found from

current year needles. Especially in situations where pollution in some area is starting it would be useful to study current year needles.

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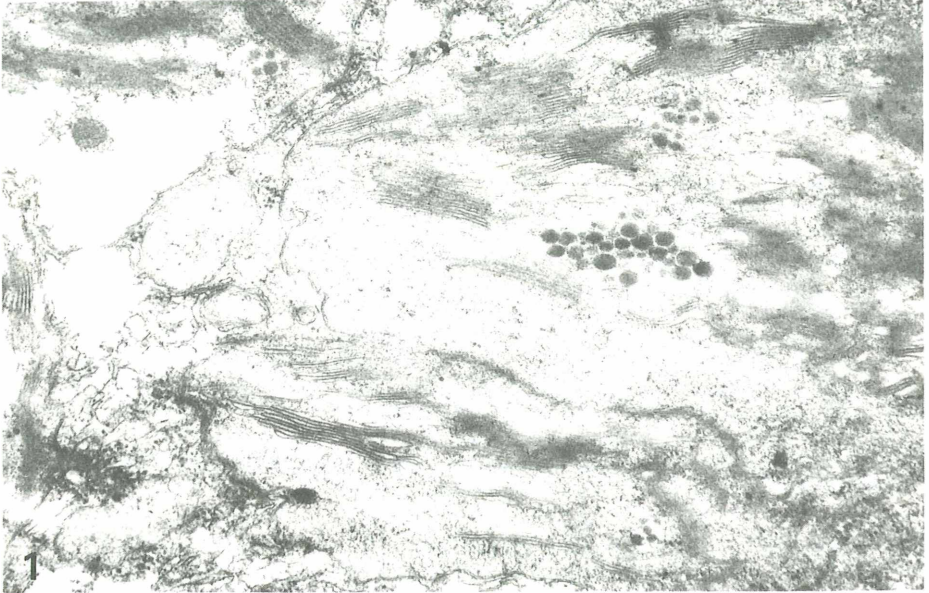


Fig. 1. Swelling of the thylakoids in a sample collected from the vicinity of a fertilizer factory. X 25 000.

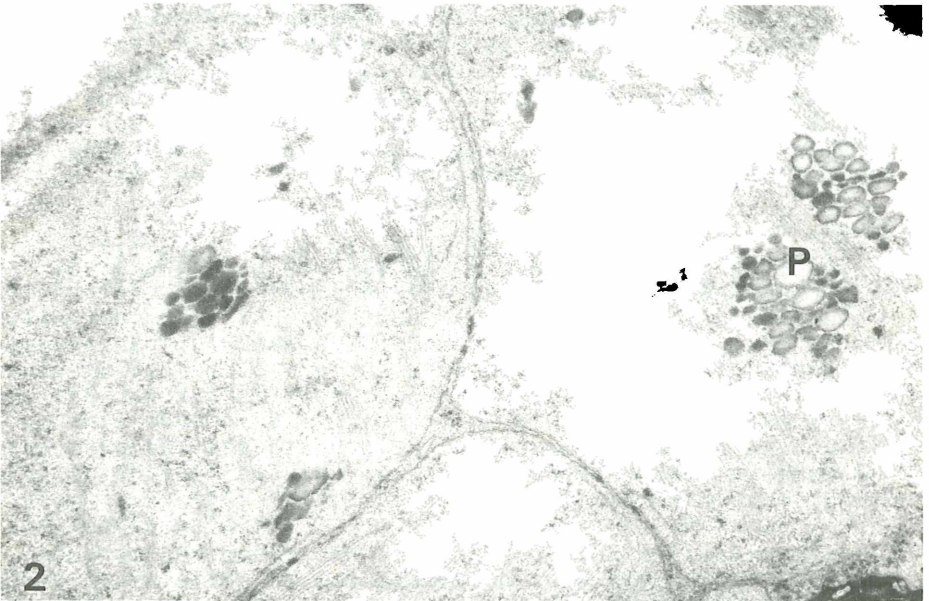


Fig. 2. Lightened plastoglobuli (P) of needle collected from the vicinity of a pulp mill. Observe also slight reduction of grana lamellae. X 39 000.

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