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Ultrastructure of chloroplasts of pine needles exposed to an industrial environment

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Abstract

In one- and two-year-old green needles of *Pinus pinaster* growing downwind from a coal-fired power station (main airborne pollutant SO_2), mesophyll chloroplast alterations consisted in swelling of the lamellae (ranging in intensity from slight to pronounced), reduction of grana number, and granulation of the stroma. The most severely affected chloroplasts were almost spherical, with highly dilated and corrugated lamellae and lacunae in the stroma. There was a large increase in the amount of lipid-like material present as droplets in cytoplasm, vacuole and stroma chloroplasts; these droplets appeared to be expulsed from the chloroplasts to the cytoplasm and vacuole. The trees with the most severely affected chloroplasts stood southwest of the power station, *i.e.* downwind with respect to the winds prevailing most of the year. Chloroplasts from two-year-old needles were more affected than those from one-year-old needles.

Introduction

Sulphur dioxide is one of the major anthropogenic pollutants produced from the combustion of coal, and the potential amount of SO_2 emitted by a coal-fire power station varies with the content of sulphur of the coal. The exposure of plants to air pollutants may cause macroscopically visible injury (chlorosis, necrosis, *etc.*). Nevertheless, before the effects of airborne pollutants become macroscopically visible, they have already damaged the physiology and ultrastructure of the plants (Soikkeli and Tuovinen 1979, Karenlampi 1986, Eversman and Sigal 1987, Miyake *et al.* 1989).

At the ultrastructural level, the first organelles affected by sulphur dioxide or its products are the mesophyll cell chloroplasts and numerous ultrastructural studies of this subject have been undertaken (Godzik and Knabe 1973, Malhotra 1976, Soikkeli 1981a, Karenlampi and Houpis 1986, Meyberg *et al.* 1988, Saastomoinen and

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Holopainen 1989). Some of the effects observed on plant chloroplasts by SO_2 is swelling of the photosynthetic lamellae, increasing the intrathylakoid space, reduction of grana lamellae (Wellburn *et al.* 1972, Soikkeli and Tuovinen 1979, Sutinen 1987b), and is often accompanied by a granulation of the chloroplast stroma (Soikkeli and Tuovinen 1979, Mlodzianowski and Bialobok 1977).

In this article we describe the alterations of the ultrastructure of mesophyll chloroplasts from *Pinus pinaster* Aiton needles growing in the vicinity of a coal-fired power station and try to delimit the influence of pollutants from other factors of stress.

Materials and methods

We studied natural sites of *P. pinaster* located in the enviroment of a coal-fire power station (1400 MW), which is situated in NW of Spain and equidistant from 30 km to the north and west Galicia coasts. The trees are growing on umbric Cambisols (FAO 1985); these acid soils (pH 4.1 - 4.7 in H_2O; pH 3.5 - 4.5 in KCl) are derived from granites and schists, possess abundant organic matter and exhibit low saturation of the exchange complex, which is dominated by aluminium.

In October, samples of one-and-two-year-old green needles were taken every 5 km along an axis stretching for 30 km on either side of a coal-fired power station in the direction of the prevailing winds (WS-NE; NE-SW), and from a control stand outside the polluted area. The main pollutant emitted by the power station is sulphur dioxide. The measurement stations, which registered the air pollutant data, are in the immediate vicinity of the sample sites. The annual average concentration of SO_2 reached values: in 1987, 10 - 18 $\mu g\ m^{-3}$ air SW, 5 - 8 $\mu g\ m^{-3}$ air NE; in 1988, 7 - 12 $\mu g\ m^{-3}$ air SW, 9 - 10 $\mu g\ m^{-3}$ air NE; in 1989, 18 - 26 $\mu g\ m^{-3}$ air SW, 6 - 11 $\mu g\ m^{-3}$ air NE. High peaks of monthly maximal values, detected by 1 h values, reached up to 500 $\mu g\ m^{-3}$, especially in summer. Quantitative data of nitrogen oxides and ozone were not available.

The needles were placed in a solution containing 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 (Karnowsky 1965), and transported in an icebox to the laboratory, where 1 mm sections were cut with a razor blade and fixed overnight in the Karnowsky solution at 4 °C. The sections were washed several times with phosphate buffer, postfixed for 4 h in a 1 % solution of OsO_4 in the same buffer at 4 °C, dehydrated through a graded ethanol series and propylene oxide, and embedded in Spurr resin (Spurr 1969). Ultrathin sections were then cut on a *Sorvall* ultratome, mounted on copper grids, stained with uranyl acetate and lead citrate, and examined under a *Hitachi H300* transmission electron microscope operating at 80 kV.

Results and discussion

Numerous studies of the fine cell structure of plants exposed to airbone pollutants (experimentally or otherwise) have shown that the first alterations occur in the chloroplasts of leaf mesophyll cells (Godzik and Knabe 1973, Soikkeli and Tuovinen 1979, Jung and Wild 1988), and they influence the photosynthetic process. However, not all the chloroplasts of a given section or cell are necessarily altered to the same extent, and not all alterations need to be apparent in all cells (Godzik and Knabe 1973, Karenlampi and Houpis 1986).

In mesophyll cells of needles from the control site we have observed the normal lobulated morphology (Esau 1965, Walles *et al.* 1973) with almost the entire cell lumen occupied by a large central vacuole filled with granular material, likely tannins (Gambles and Dengler 1982). The cytoplasm, bound by plasmalemma and tonoplast, contained the usual organelles, including a single layer of chloroplasts at the cell boundary. Electron microscopy showed lenticular chloroplasts containing well-developed internal membrane systems with densely piled thylakoids (Fig. 1a,b). Their stroma was homogeneous and had few electron-dense plastoglobules. Lipid-like droplets were observed occasionally in chloroplasts, cytoplasm and vacuole.

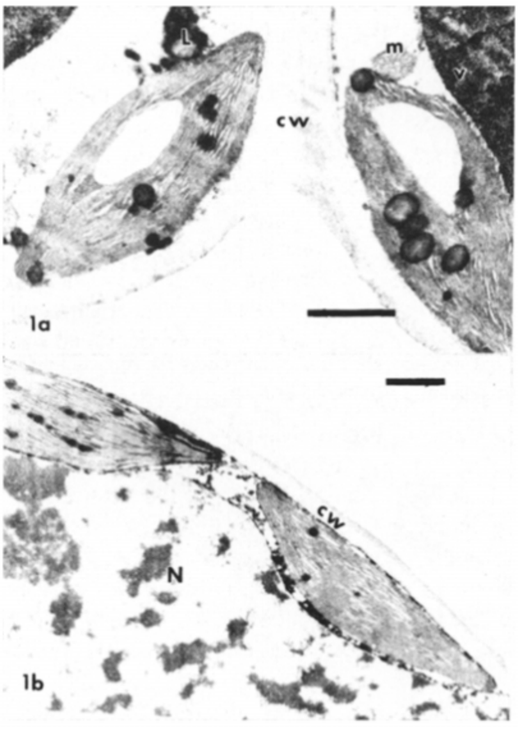

Fig. 1a,b. Mesophyll chloroplasts of needles from control trees. The stroma chloroplast is light and grana stacks are high. The content of the central vacuole appears as a large granular mass. The abbreviations used in the electron micrographs: Cw = cell wall; L = lipid; m = mitochondrion; N = nucleus; Pg = plastoglobules; Sg = starch grain; Th = thylakoids; V = vacuole. Bar = 1 μm.

Samples from WSW of the power station: Mesophyll cells of two-year-old green needles collected at all sampling points southwest of the power station contained altered chloroplasts. The chloroplasts tended to become rounded and their photosynthetic lamellae exhibited various degree of swelling, with fewer, smaller grana (Fig. 2). The most severe damage was represented by rounded chloroplasts with strong swollen thylakoids and increased granulation of the stroma, light plastoglobules, and high content of lipid-like droplets in the cytoplasm (Fig. 3).

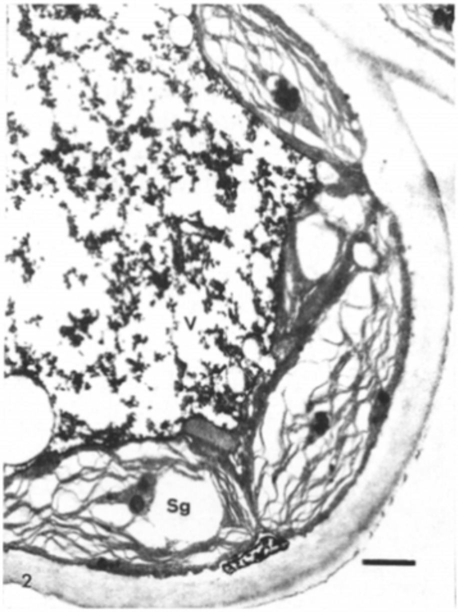

Fig. 2. Mesophyll chloroplasts of two-year-old needles collected 20 km southwest from the power station. Corrugated photosynthetic membranes and scant stroma can be seen.

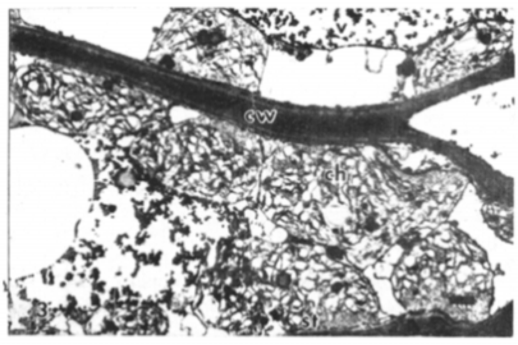

Fig. 3. Part of a mesophyll cell of two-year-old needles in samples taken southwest of the power station. The thylakoids are severely swollen and the stroma shows electron-transparent gaps.

Soikkeli (1981a) and Soikkeli and Tuovinen (1979) reported the same alterations in mesophyll cells of pine and spruce needles growing in industrial areas in which SO_2 was the main airborne pollutant. Similar changes were observed by Karenlampi and Houpis (1986) in *Pinus ponderosa* plantlets fumigated for two years with 75 or 150 $\mu g\ kg^{-1}$.

Although slight swelling of thylakoids has been sometimes seen in chloroplasts of needles of healthy trees (Soikkeli 1981a, Ebel *et al.* 1990), and even at certain stages of the normal life cycle of plastids (Senser *et al.* 1975), strong swelling of the lumen spaces within the thylakoids and the formation of electron-transparent gaps in stroma have been observed practically in all the experiments where SO_2 is involved (Wellburn *et al.* 1972, Miyake *et al.* 1984, Forschner *et al.* 1989). The granulation of stroma has been observed by Soikkeli and Tuovinen (1979) and Soikkeli (1981a) in *Picea abies* and *Pinus sylvestris* growing in S-polluted areas; however, this effect were found in severely damaged cells, as well.

Besides these alterations, other types of chloroplasts were observed in two-year-old needles collected 15 km from the power station (Fig. 4). They were elongated, with dense, uniform and dark stroma, and generally occurred in cells whose other chloroplasts were severely altered. This increase in the density of stroma has also been observed by Swanson *et al.* (1973) after ozone fumigation.

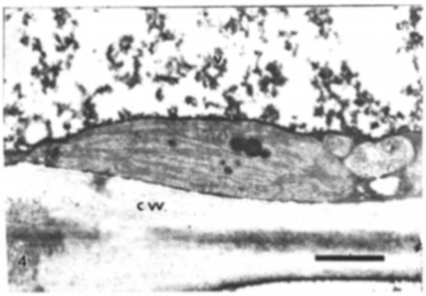

Fig. 4. Mesophyll chloroplasts of two-year-old needles collected 15 km southwest of the power station. Reduction in grana stacking (*arrow*) and an increase of osmiophilia in the stroma can be seen. Tannins in central vacuole (V) are distributed as disperse granules.

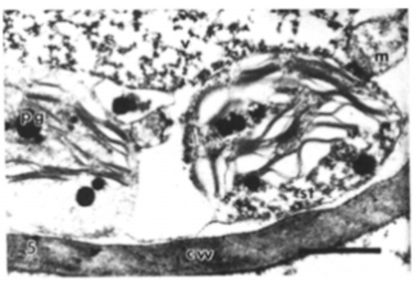

Fig. 5. Rounded chloroplasts of two-year-old needles taken in the southwest direction of the central power. Note an increase in the granulation of the stroma and strongly swollen lamellae (*arrow*). Mitochondria membranes are poorly defined.

No specific alteration of endoplasmic reticulum, Golgi complex or other organelles was observed, but mitochondria had sometimes a diffuse appearance. Mitochondria membranes were sometimes poor defined in sections of cells in which the chloroplasts were mostly severely altered (Fig. 5). Soikkeli (1981a) regarded as severely damaged those mesophyll cells that exhibited not only damaged chloroplasts but also altered cytoplasm and cytoplasmatic organelles.

The content of the central vacuole, presumibly tannins (Soikkeli 1981a, Sutinen 1987a), usually appears as disperse granules or as a large granular mass. The cytoplasm generally exhibited numerous vacuoles or vesicles.

Most cells contained abundant lipid-like droplets in chloroplasts, cytoplasm and vacuole, and we have seen an expulsion of these droplets from chloroplasts into the cytoplasm, in samples taken at 5 and 20 km in both wind directions (Fig. 6). This expulsion has been described by Miyake *et al.* (1989) in radish plants fumigated with 0.05 cm(O_3) m^{-3} for 24 h a day for 6 d. At the same time, the plastoglobules become larger and lighter, and this decrease in affinity to osmium tetroxide may be due to a peroxidation of the unsaturated lipids in the thylakoids. This peroxidation is induced by free radicals, which are produced by SO_2 or O_3.

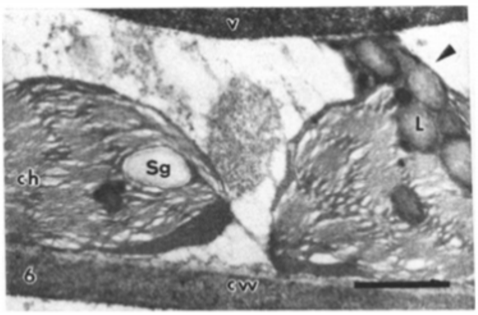

Fig. 6. Lipid-like droplets apparently being expulsed from the mesophyll chloroplast of one-year-old needles.

Starch was scarce, most chloroplasts contained no more than one small-to-medium sized grain and the most severely altered chloroplasts either no starch at all or just a very small grain.

Similar findings were obtained for one-year-old needles, *i.e.* chloroplasts with swollen photosynthetic lamellae, fewer grana, and lighter plastoglobules; but the shape of the chloroplasts did not change and the swelling of the thylakoids was not so strong as in two-year-old needles. In one-year-old needles, we could also see the expulsion of lighter plastoglobules from chloroplasts into cytoplasm.

Samples from ENE of the power station: Chloroplast alterations in needles collected northeast of the power station at distances of 5 and 30 km were similar to those described above, except that no cases of severe damage were observed; thylakoids were corrugated and slightly swollen (Fig. 7), but the shape of chloroplasts was not modified (Fig. 8). Chloroplasts of both one-and-two-year-old needles from intermediate sampling points located from 15-20 km of the power station, had unaltered membrane systems, but contained starch grains that were almost as large as in spring (unpublished data) (Fig. 9); whereas at all other sampling points, *i.e.* WSW, ENE and controls, starch grains were smaller in October than in spring. Findings on the effects of pollutants on starch metabolism have been contradictory. Saastomoinen and Holopainen (1989) found that the only chloroplast alterations in SO_2-fumigated *Pinus sylvestris* plantlets were swollen thylakoids and a reduction in the size of starch grain. Forschner *et al.* (1989) suggested that starch accumulation in mesophyll cells of two-year-old spruce needles may be due to pollution by O_3 or O_3/SO_2 mixtures, and that soil nutrient deficiencies may also play a role. Ebel *et al.* (1990)

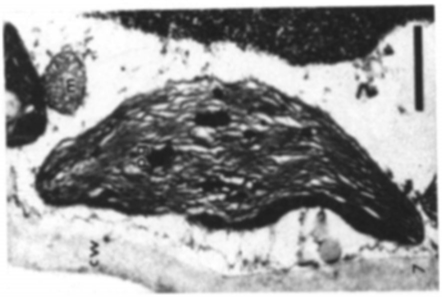

Fig. 7. Mesophyll chloroplasts of one-year-old needles taken at the northeast of the power station. Tightly corrugated lamellae.

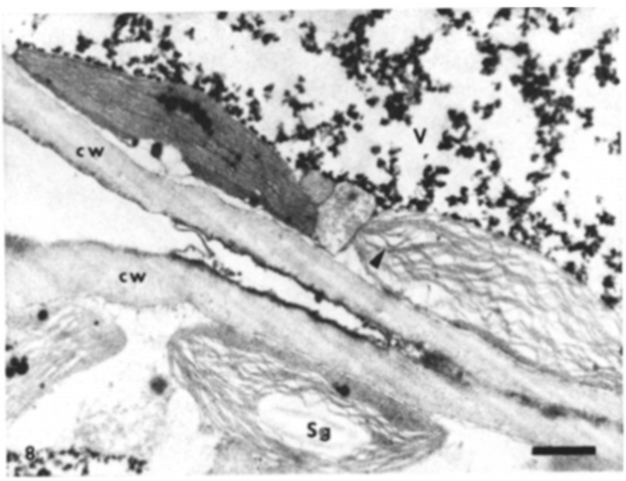

Fig. 8. Mesophyll cells of one-year-old needles collected at the NE of the power station. Both damaged and undamaged chloroplasts can be seen. Note the corrugated lamellae in the damaged chloroplasts (*arrow*).

found a starch accumulation in mesophyll cell in a long-term fumigation with O_3/acid mist in young spruce trees. In needles taken at the northeast of the power station the large size of the starch grains may have been due to delayed mobilization caused by any of the reasons mentioned above.

Lipid-like droplets in the cytoplasm were fewer than in samples taken in the southwest of the power station, and were scarce in chloroplasts and vacuole. The tannins of the central vacuole exhibited coarse and/or fine granular texture (Soikkeli and Tuovinen 1979, Ebel *et al.* 1990).

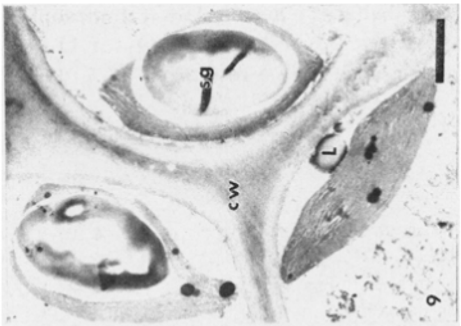

Fig. 9. Large starch grains in otherwise normal chloroplasts of two-year-old needles collected 20 km from the central power at the northeast direction.

As regards the degree of alteration reached by one-and two-years-old needles taken from the NE of the power station, no relevant differences have been due to the lowest levels of emission of SO_2 recorded in this area, in relation to their SW location.

Since the forest decline is due to multiple factors rather than one single cause, recent research has attempted to distinguish between the effects of airborne pollutants and those of other biotic and abiotic variables such as bioclimatic, pedological and phytopathological conditions. Reinikainen and Huttunen (1989), for instance, found that in the needles of pine and spruce plantlets subjected to artificial acid rain and low temperatures, the initial effect of frost was excessive cell dehydration and consequent compartmentation of cytoplasm, whereas pollutants initially affected chloroplasts. Holopainen and Holopainen (1988) observed that the cytoplasm of the cells of needles of *Pinus sylvestris* plantlets was affected by summer frosts simulated in growth chambers, but chloroplast structure was more resistant. The results of growth chamber studies are not necessarily extrapolable to field conditions, since the physiological responses of forest stand trees can differ from those of plantlets or saplings; in natural habitats the oldest needles are more susceptible to damage, whereas in growth chamber studies are the youngest needles (Soikkeli 1981b, Frank and Frank 1986, Barnes and Davison 1988).

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