Reactions of Plant Cells on Air Pollution*)

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With 8 Figures

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Summary


Air borne pollutants first reach the surfaces of plants. On the leaves the pollutants influence the cuticular area and epicuticular waxes. Then the air pollutants enter the leaf through the stomata. They dissolve in the cell wall water. Concerning the cell itself pollutants effect the membranes and the plasmatic layer. Ultrastructural investigations show significant changes in the chloroplasts of green and yellowed needles from different polluted areas. The damages mainly affect the thylakoid-system and the plastoglobuli but changes also occur in the carbohydrate metabolism. Air pollutants cause a different course of damage in the needles than nutrient deficiencies do. This could be demonstrated also by different fumigation experiments.

Zusammenfassung


Luftgetragene Schadstoffe erreichen zuallererst die Oberfläche von Pflanzen. Besonders an den Blättern werden die Kutikula und die epikutikulären Wachse

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Introduction

Soon after macroscopic consequences of air pollutions on plants, such as altered growth and discolouration or yellowing of the leaves, were discovered at the end of the 19th century, research started to investigate also the changes in the microscopical range. These first investigations were mainly done with necrotic objects and were not this important for finding the causality. SCHRODER & REUSS 1883, HARTIG 1896, HASELHOFF & LINDAU 1903, WIETER 1905, LOEB 1909, SORAUER 1911, NEGER & LAKON 1914, WISLICENUS 1914, HASELHOFF & al. 1932, ANDRE 1934, LEPESCHKIN 1937, THUMLER 1942 just to mention a few, did a lot of research concerning this field, whereby the role of SO$_2$ was focused. So it became possible to draw a clear picture of severely damaged cells. They talked of a granular structure of the plasma, a vacuolisation, a detachment of the plasma membrane from the cell wall, disintegrated organelles and coagulation of the cytoplasm at the end. However, when you wanted to know how air pollutions affect a cell, the investigations mentioned above did not elucidate anything. Although these reports dealing with the cell-structure of damaged plants belong to the earliest findings done in air pollution research, cell physiology nearly neglected this field for a long time. The biochemical-physiological field was favoured. That is quite surprising, because cell physiology combined with electron microscopical methods reveals a realistic and complete picture of the way a plant cell reacts to stress.

SEM-Investigations

The influence of air pollutants on the cuticular area was studied only a little by using TEM, but a lot with SEM. However, only a few investigations cover the cuticula itself. E. g. GODZIK & HALBWACHS 1986 discovered that the folds of the cuticula disappear under the influence of emissions produced by heavy metal factories. Most of the papers deal with the epicuticular wax, mainly those of conifers. The needles of conifers have lots of little wax rodlets on the surface as well as covering the stomata. Since the first investigations in the early seventies (GRILL 1973, BLIGNY & al. 1973) it was often reported that acid air pollutions, especially SO$_2$, cause to fuse
Figs. 1–4. SEM-investigations, *Picea abies*, wax rods in the stomatal antechambers; bar = 1.68 \( \mu \)m.

Fig. 1. Uninfluenced wax rods that compose a dense meshwork.

Fig. 2. Beginning influence, the wax rods fuse at their tips and larger pores in the dense meshwork of the wax rods become visible.

Fig. 3. Enhanced influence, advanced dispersion of the dense mesh of wax rods.

Fig. 4. Very strongly influenced wax structures, the crystalline structure is lost and the stoma is occluded with amorphous wax.

precipitation wax destructions are found too (KAZDA & GLATZEL 1986, RINALLO & al. 1986, SCHMITT & al. 1987). Also aerosols (KRAUSE 1982) and particularly alkaline dusts (GRILL & GOLOB 1983, BERMADINGER & al. 1987, 1988a) are thought to have a persistent effect on waxes. Ozone (TRIMBLE & al. 1982, GÜNTHARDT-GOERG & KELLER 1987) respectively new type damaged spruce needles (BERMADINGER & al. 1988b) have no effects on waxes, neither do neutral dusts. (GRILL & GOLOB 1983). But BYSTRON & al. 1968, BARNES & al. 1988 found alterations of the wax structure at high ozone concentrations. The detailed causes of wax destruction are still unknown. According to JEFFREE & al. 1971 waxes strongly influence the gas exchange: in contrast to a wax-free antechamber the stomatal diffusion of water vapour is reduced by 2/3 but that of CO$_2$ only by 1/3. Therefore changes of the wax structure imply consequences. The first stage is a formation of cavities between the wax crystals caused by the onset of a fusion of the wax rodlets (Fig. 1–3). As a consequence the stomatal conductance for water vapour increases (BERMADINGER & GRILL 1987). The next stage leads to a more extensive fusion and in the end to a complete occlusion of the stomata by amorphous wax (Fig. 4) (BERMADINGER & al. 1987); the result is a CO$_2$ shortage (BERMADINGER & GRILL 1987). HUTTUNEN & al. 1981 suppose that cuticular transpiration can no longer be controlled if the wax is eroded. A larger wettability is due to the erosion of wax on the leaf surface (CAPE 1983). Through it the possibility of an infection by pathogens is even bigger (ELSTNER & OSSWALD 1984, MATHE 1985). The range of wax destruction depends on the vitality of the tree: the higher the vitality, the smaller the wax destruction (GRILL & al. 1987).

**Physiological Reactions**

The largest amount of air pollutants enter the leaf through the stomata. That is why the cells of the stomata are extremely endangered (GARSED & MOCHRIE 1980, HEATH 1980, GARSED 1984, MALHOTRA & KHAN 1984, PARRY & WHITTINGHAM 1984). After the pollutants have entered the leaves they dissolve in the cell wall water. Concerning the cell itself pollutants effect first the plasmic layer.

When using quite high acid concentrations in short time experiments it was found that the mortality rate caused by H$_2$SO$_3$ and other acids does not differ. However, using low concentrations in long time experiments, sulphurous acid is many times more toxic than for example sulphuric acid. The degree of toxicity, this means the consecutive reactions in the cell, depends on the object, the season, the age, but also on the type of the affected tissue (HÄRTEL & MIKLAU-GRASSL 1974, GRILL & HÄRTEL 1972 – see also HÄRTEL 1986).

The ability of cells to hold water is reduced significantly at a concentration of not more than 0.1 mg SO$_2$ respectively sulphurous acid with a
concentration of 3.4 μmol. Disorders in the boundary layer of the plasma are responsible for the altered permeability, but not a changed osmotic value.

Besides the effect on the plasma-membranes, also the effect on the plasma itself has to be discussed. That is indicated by the concave shape after plasmolysis on one hand and concentration dependant alterations of the plasmic-streaming on the other hand. Detailed data are to be found in a paper by Härtel & Miklau-Grassl 1974, in which also further investigations on this topic are mentioned.

Härtel & Miklau-Grassl 1974 thought – already in those days – that the thiol-groups of proteins were the first target when SO₂ is the toxic compound. Thereby, depending on the concentration a more or less strong sulphotolysis of disulphide compounds has to be expected. In addition to that and even predominant with lower SO₂ concentrations also an increased amount of sulfhydryl-compounds (-SH), for example glutathione, was expected caused by an increased metabolism of sulphite respectively sulphate. A high amount of glutathione can also lead to a shifting of the SH/SS relation in cell proteins (Rennenberg 1984). A long lasting SO₂ emission is not only the reason for a higher amount of glutathione but also of protein-SH (Pfeifhofer & al. 1988). Acid air pollutants reach the plasmalemma and penetrate it undissociated. Then according to the pH of the cell the molecules dissociate. Thereby the neutral cytoplasma and the alkaline irradiated chloroplasts have a higher buffer capacity than the acid vacuoles. The acid therefore dissociates according to its chemical nature especially in the plasmic and plastidic area, resulting in a flow from outside into the cell. The faster the cell scavenges both the anion and the proton the more it withstands such pollutants. According to the work of Laisk & al. 1988a, 1988b it matters how fast bisulphite respectively sulphite are oxidized into sulphate whereby the formation of radicals is assumed (Jäger & Klein 1977, 1980). On the other hand, it is important how fast the cell can get rid of the protons which are present in sulphite too. Therefore the most dangerous insults affect the alkaline chloroplasts and that is supposed to be one important reason for the reduced photosynthesis.

An essential detoxification mechanism is the reduction of bisulphite respectively sulphite into sulphide; it is even more important than the storage capacity of the vacuoles for sulphite and protons. In this reaction the anion takes part and protons are used up. Pfanz & HEBER 1986 indicate that SO₂ concentrations which are just below being considered to be dangerous can exhaust the vacuole's storage interfere. Therefore it is important for conifers having quite acid vacuoles.

That is why plants having a high thiol turn over rate (for example fast growing plants) might be more resistant than trees. Here the portion of reduced sulphur is small. In this case, according to Pfanz & al. 1978, already 0.05 ppm SO₂ could be toxic, when air pollution lasts for a long time. Finally they pointed out that, according to their computer analysis
based on chemical data, the main barrier for \( \text{SO}_2 \) penetration into leaves are the stomata. Biomembranes represent only a minor barrier. So the detoxification of \( \text{NO}_2 \) and \( \text{HF} \) is to be seen. However KRONBERGER 1987 points out that because fluoride cannot be metabolised in the plant it is extremely toxic and it accumulates in the cytoplasm respectively in the plastides. Investigation done on organic lead compounds showed an effect on the water permeability already at the very low concentration of 0.1 \( \mu \text{mol} \) (GUTTENBERGER & al. 1988). In consequence, it was found that membranes change their permeability for ions, especially for chloride.

HAGER & al. 1987 found out that triethyl lead (TriEL) acts as an anion antiporter in plant membranes, dissipating energy-dependent ion gradients, membrane potentials and consequently turgor. This mechanism was demonstrated with tonoplast-type vesicles isolated from maize coleoptiles.

The ATP-driven \( \text{H}^+ \) accumulation within those vesicles was abolished already at nano-molar levels of TriEL. With concentrations higher than 1 \( \mu \text{M} \) an additional inhibitory effect of TriEL was observed: the tonoplast-type \( \text{H}^+ \)-pump activity depends on regulatory thiol groups on the enzyme.

\( \text{Et}_3\text{Pb}^+ \) (TriEL) interacts with these thiols of the \( \text{H}^+ \) ATPase; a re-reduction by glutathione restores the activity. But this SH-blocking effect might not be of importance under in vivo conditions because TriEL already disturbs cell metabolism in a much lower, nanomolar concentration range. Supposed that the more sensitive a mechanism reacts to insults the more it is of importance to the cell-life. Than it seems that these osmotic processes respectively processes at the membranes are one of the most important targets for pollutants. According to HOCK & ELSTNER 1988 oxidants first effect unsaturated membraneous fatty acids. In a chain-reaction free radicals transform fatty acid into hydrocarbons, aldehyds or alcohols. The current differences in fatty acid composition in plants, insects, higher developed animals and so on could be connected with different \( \text{O}_3 \) content in the atmosphere at different phylogenetical periods. Thus HOCK & ELSTNER 1988 explain the different sensitivity of various organisms concerning the insults done by \( \text{NO}_2 \) and \( \text{O}_3 \). New methods in cell biology, for example fluorescence microscopy used after indirect immunreacion, UV microscopy or the AVEC-DIC (Allen Video Enhanced Contrast Differential Interference Contrast, ALLEN & al. 1981b) or AVEC-POL (Video Enhanced Contrast Polarisation, ALLEN & al. 1981a) method connected with computer (ALLEN & ALLEN 1983), just to mention a few, are modified to meet the new challenges in the research of emissions, so that new facts can be revealed and a connection between light- and electronmicroscopy can be achieved.

TEM investigations

Electron microscopical investigations concerning the effects of air pollutions on plants are numerous. Many investigations done on conifers
lead to a lot of new aspects. Air pollutants cause ultrastructural changes in mesophyll cells especially in chloroplasts. MEYBERG & al. 1988 reported that in damaged spruce needles from Northern Germany only chloroplasts were affected, but not the nucleus, mitochondria, microbodies, or the endomembrane system of the cell. With the spruces we have examined, significant changes were already found in chloroplasts of one-year-old green needles from SO$_2$ and “new type” damaged areas. These changes were obviously even more distinct with three-years-old green needles; they mainly show disintegration of chloroplasts, alterations of the thylakoid-system and of the plastoglobuli. Here, it should be mentioned that in spruce-chloroplasts the natural aging process starts not before the fourth year. All changes which can be detected before have to be regarded as abnormal. However, the theories about the senescence of chloroplasts in spruces differ. MEYBERG & al. 1988 think increased plastoglobuli number and membrane reduction to be characteristic, while SUTINEN 1987a described a change of the shape of chloroplasts, a few electron-light plastoglobuli and a good developed membrane system in connection with senescence. Besides changes caused by pollutants differ from the ones caused by the normal aging process. Principally we know that certain substances, such as SO$_2$ or HF cause the so called “smoke damages”. Different papers nearly go conform in describing the structural changes associated with those damages, but the changes often get differently combined. MALHOTRA 1976 reported mainly about the enlargement of thylakoids with Pinus, while SOIKKELI 1981 points out lightening of plastoglobuli and swellings of thylakoids under the influence of SO$_2$. Also SO$_2$ fumigation experiments with spinach leaves caused an enlargement of thylakoids and a deformation of chloroplasts (MIYAKE & al. 1984). When we examined “SO$_2$” damaged spruce needles, we detected similar symptoms like plastoglobuli lightening, increase in the number of plastoglobuli and enlargements of the intrathylakoidal space (Fig. 5).

With the “new type” damaged spruces it was not possible to find coherent symptoms. The variety of structural damages is even larger (cf. JUNG & WILD 1988). This might, most of all, be due to different pollutions of the so called “oxidative type”. Nevertheless, comparing “new type” damaged needles with the “SO$_2$” damaged ones, differences were observed. When we examined chloroplasts of one-year-old green needles of “new type” damaged spruces, we mainly found enlargements of thylakoids. With three-years-old ones we registered an increased number of sometimes inhomogeneously stained plastoglobuli and a reduction of grana thylakoids (Fig. 6). In yellowed needles a reduction of the membranes and an accumulation of starch takes place already in the first year needles. In addition to these symptoms the third-year needles show a strong accumulation of plastoglobuli (Fig. 7). SUTINEN 1987b describes similar changes with yellowed needles from the Taunus area (FRG), which might be caused by longer lasting influences of air pollutants like SO$_2$+O$_3$. These symptoms seem to be
characteristic for “new type” damaged spruces, while electron-light plastoglobuli and severe changes of the chloroplast shape are found in “SO$_2$” damaged spruces.

The changes of the plastoglobuli as well as the accumulation of starch in the chloroplasts indicate disorders respectively alterations of the lipid- and carbohydrate metabolism. Thereby, the differences in the electron opacity of plastoglobuli express an altered composition of the lipids (Lichtenthaler 1970). Lichtenthaler & Buschmann 1984 pointed out that the enlargement of the intrathylakoidal space is a sign for modification at the molecular level. Thus the normal function of this membrane gets disturbed. After the research we did on chloroplasts of SO$_2$ and “new type” damaged spruces and pointing out diverse symptoms, we also examined one- and three-years-old green needles of spruces taken from regions with low air pollution. Thereby some interesting news got revealed. Besides normal chloroplasts, also some with an increased number of plastoglobuli, enlarged intrathylakoidal spaces and altered contours could be detected (Fig. 8). Because of these alterations it is now very important to find the reason for such symptoms. To do so, different kinds of fumigation experiments are carried out. In the meantime the applied concentrations are adjusted near to reality so that comparisons of ultrastructural changes are much more realistic (cf. Ruette & al. 1988). On the other hand, also the nutrition status is of great interest. For example, a lack of iron also can cause enlargements of the thylakoids and a lack of magnesium an accumulation of starch (cf. Fink 1988). Besides this, heavy metals which are known to cause damages in various water plants, could get through the soil and the root system into the plant. But the heavy metal induced damages have a larger variety and affect not only chloroplasts, but also nuclei, mitochondria, endoplasmatic reticulum and the cell wall structures (Röderer 1986, Heumann 1987). Such

Figs. 5–8. TEM-investigations, Picea abies, mesophyll-cells; bar = 1 μm.

Fig. 5. First year green needle from a SO$_2$ polluted area; chloroplast with inhomogeneously stained, often lightened plastoglobuli (P), swellings of the intrathylakoidal space (arrows) and a membrane-free stroma space (*).

Fig. 6. Third year green needle from a “new type” damaged region; chloroplast with increased number of plastoglobuli (P) and a reduced thylakoid-system; starch (S).

Fig. 7. Third year yellowed needle from a “new type” damaged region; chloroplast shows strong deformations, a high amount of electron opaque plastoglobuli (P) and only few thylakoids (arrows); starch (S).

Fig. 8. Third year green needle from a low air polluted region; chloroplast with a high amount of electron opaque plastoglobuli (P) and a membrane-free stroma space (*).
kinds of pollutions can not only be detected by examining the soil, but also by the different course of damage in the needles. Here the symptoms first occur in the vascular bundle area; later, the damages are also seen in the mesophyll cells. On the contrary, gaseous pollutants first affect the mesophyll cells. This was obvious in fumigation experiments which revealed damages only in the stomatal area and in the mesophyll but never in the vascular bundle. Besides, damaged chloroplasts of mesophyll cells show gradually decreasing structural alterations from the parietal face towards the center (RUETZE & al. 1988). To the ultrastructural research the analysis of the pigments is added. The advantage of this is the better way of quantification, of whatever you could already detect by electron microscopical means. The knowledge about the annual rhythm and the dependency of the age is important. Thus, interesting results can be achieved, whereby the relation of the various pigments to each other is more informative then the total amount, as PFEIFFHOFER & GRILL 1987 have mentioned. For example, a change of the relation of xanthophyll to carotene indicates oxidative processes, but the appearance of zeaxanthin shows troubles of the electron flow in the course of photosynthesis.

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