ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVI, nr 4
1977

The effect of sulphur dioxide on ultrastructural organization of larch needles*

F. MŁODZIANOWSKI AND S. BIAŁOBOK

Institute of Biology, A. Mickiewicz University, Poznań
Institute of Dendrology, Polish Academy of Sciences, Kórnik

(Received: June 15, 1977)

Abstract

Several changes have been observed in the ultrastructure of the mesophyll of larch needles under the effect of SO_2: swelling and degradation of the thylakoids, granulation of the cytoplasm and plastid matrix, degradation of the ribosomes and of endoplasmic reticulum, agglutination of the chromatin, and cell plasmolysis. Comparison of the ultrastructure in two larch-trees characterized by different susceptibility to SO_2 appeared to be difficult due to the lack of synchronousness in the reaction of particular cells to this gas.

INTRODUCTION

Sulphur dioxide is one of the factors polluting the air. It can cause several irreversible changes in plants (Saunders and Wood 1973). On the other hand it is known that sulphur is necessary for normal plant metabolism. According to Treshow (1970) sulphur content in green leaves varies between 0.5 and 14 mg/g of dry weight. Concentrations below 0.25 mg/g of dry weight are critical, resulting in symptoms characteristic of the deficiency of this element.

Part of the sulphur content of plants may originate from atmospheric SO_2 if its concentration in the air is not too high (Ross and Clarke 1973). If the concentration of SO_2 in the air increases above normal level several metabolic processes of plants become inhibited, and especially the photosynthesis. According to Godzik (1976) present state of our knowledge allows for stating that negative effect of SO_2 on plants is

* This work has been partially supported by grant FG — Po — 326 from the US Department of Agriculture.

much stronger than its possible fertilizing action. Significant increase of SO_2 concentration in the atmosphere, together with prolonged exposition of plants to this gas, can lead into irreversible changes, or even to the death of cells, organs, or the whole plant. Nevertheless, tolerance of plants to SO_2 depends on many factors connected with the plant itself, as well as on environmental conditions (Godzik 1976).

Destructive effect of smoke upon chloroplasts has been noted already at the turn of the 19th and 20th centuries (Sorauer and Ramann 1899, Haselhof and Lindau 1903). Hölte (1958) showed that, apart from plasmolysis and dehydration of cells, SO_2 caused significant deformation of chloroplasts.

Among many studies on the effect of various harmfull factors on the mesophyll, carried out taking advantage of electron microscopy, only a few delt with the effect of SO_2.

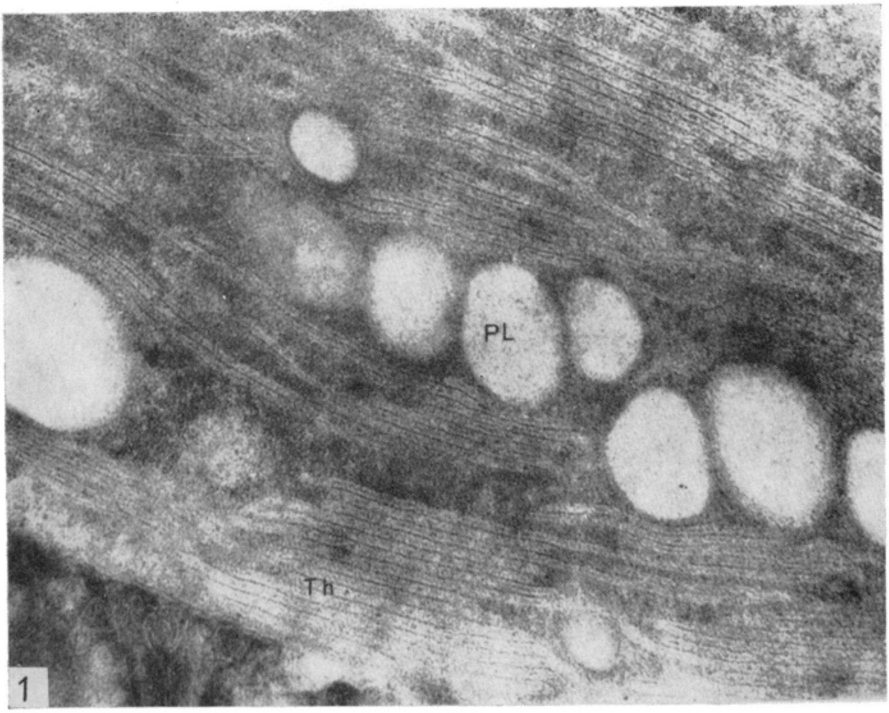
Fischer (1967), Fischer et al. (1973), and Wellburn et al. (1972) described changes caused by SO_2 in *Vicia faba*, Godzik and Sassen (1974) — in *Phaseolus vulgaris*. Godzik and Knabe (1973) undertook comparative studies on some species of pine trees from industrial districts of the German Federal Republic, and Malhotra (1976) described ultrastructural and biochemical changes in the needles of Scotch pine-tree treated with the aqueous SO_2.

Destructive changes of chloroplasts under the influence of SO_2 are accompanied by a decrease of photosynthetic activity. Photosynthesis is one of the first processes disturbed by the effect of SO_2 (Malhotra and Hocking 1976). Decreased level of photosynthesis was observed in many plants (Lüttke and Fischer 1971, Puckett 1974, Inglis and Hill 1972, Ziegler 1975), as also in *Larix leptolepis* (Lorenc-Plucińska 1976). In the latter case individuals susceptible and resistant to SO_2 were obtained by selection. Individuals defined as resistant assimilated during the photosynthesis three times as much CO_2 in the presence of SO_2 as the susceptible ones (Białobok 1976).

It seemed that it would be interesting to obtain more knowledge on the mesophyll ultrastructure of these individuals, the more so that some differences in the structure of photosynthetic apparatus might have been expected.

MATERIAL AND METHODS

Studies were carried out on two selected Japanese larch trees *(Larix leptolepis)* characterized by different susceptibility to SO_2 (Białobok 1976). Twigs cut from both trees were placed in water. Next, part of them was placed in a special chambers and treated with SO_2 in the concentration of 2.5 ppm for 8 hours daily, during three days (22 — 24



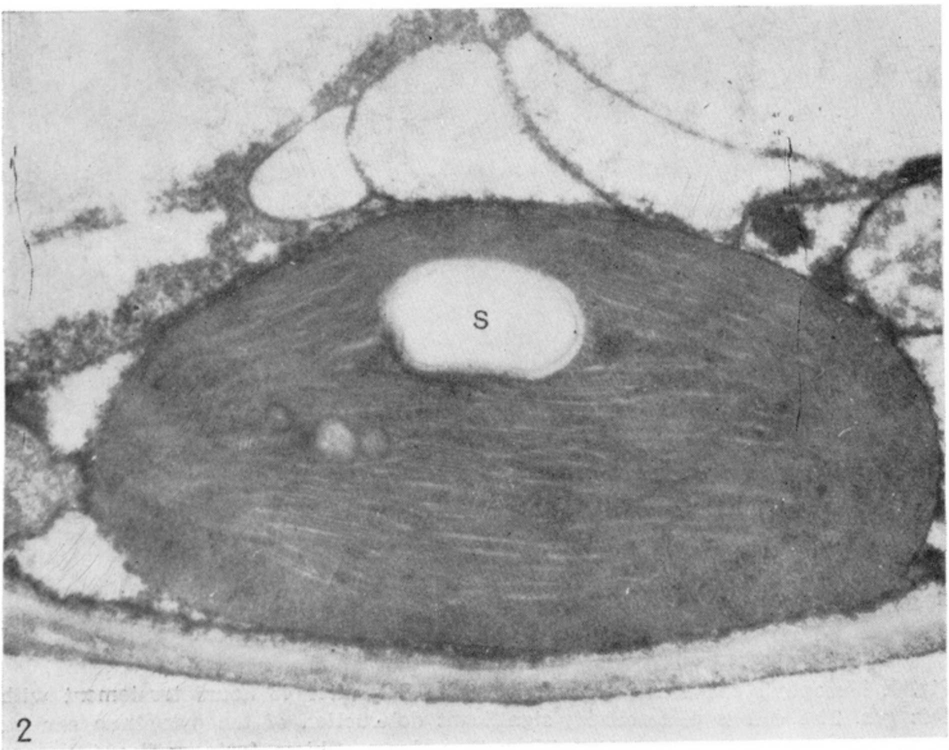


Fig. 1. Part of a chloroplast from initial material obtained from larch resistant to SO_2. Visible well preserved structure with regular distribution of thylakoids (TH) and plastoglobules (Pl.) ×50 000

Fig. 2. Chloroplast with slightly swollen thylakoids and preserved starch (S). in larch needle of resistant tree after 48 hours of experiment (control)

Fig. 3. Mesophyll cell from larch resistant to SO_2 after 48 hours treatement with this gas. The cell is plasmolized; significant destruction of the cytoplasm can be seen. Chromatin in the nucleus (N) rather dense. Chloroplast profiles (CH) relatively well preserved, with visible starch grains (S). Thylakoids invisible. ×5 000

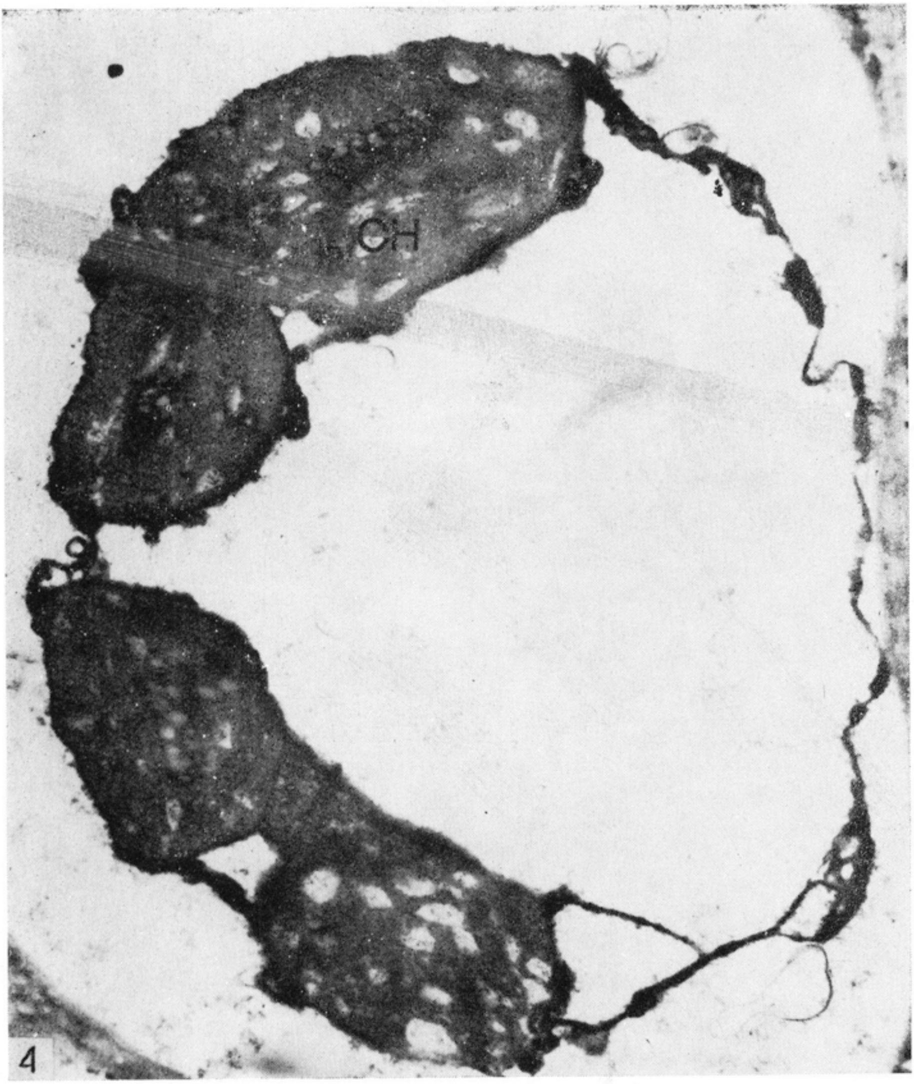


Fig. 4. More advanced than in Fig. 3 degradation of cell induced by SO_2 treatement in larch resistant to this gas. Total destruction of the cytoplasm in significantly plasmolized cell. No thylakoids in the chloroplasts (CH) but their matrix well preserved. ×8 000

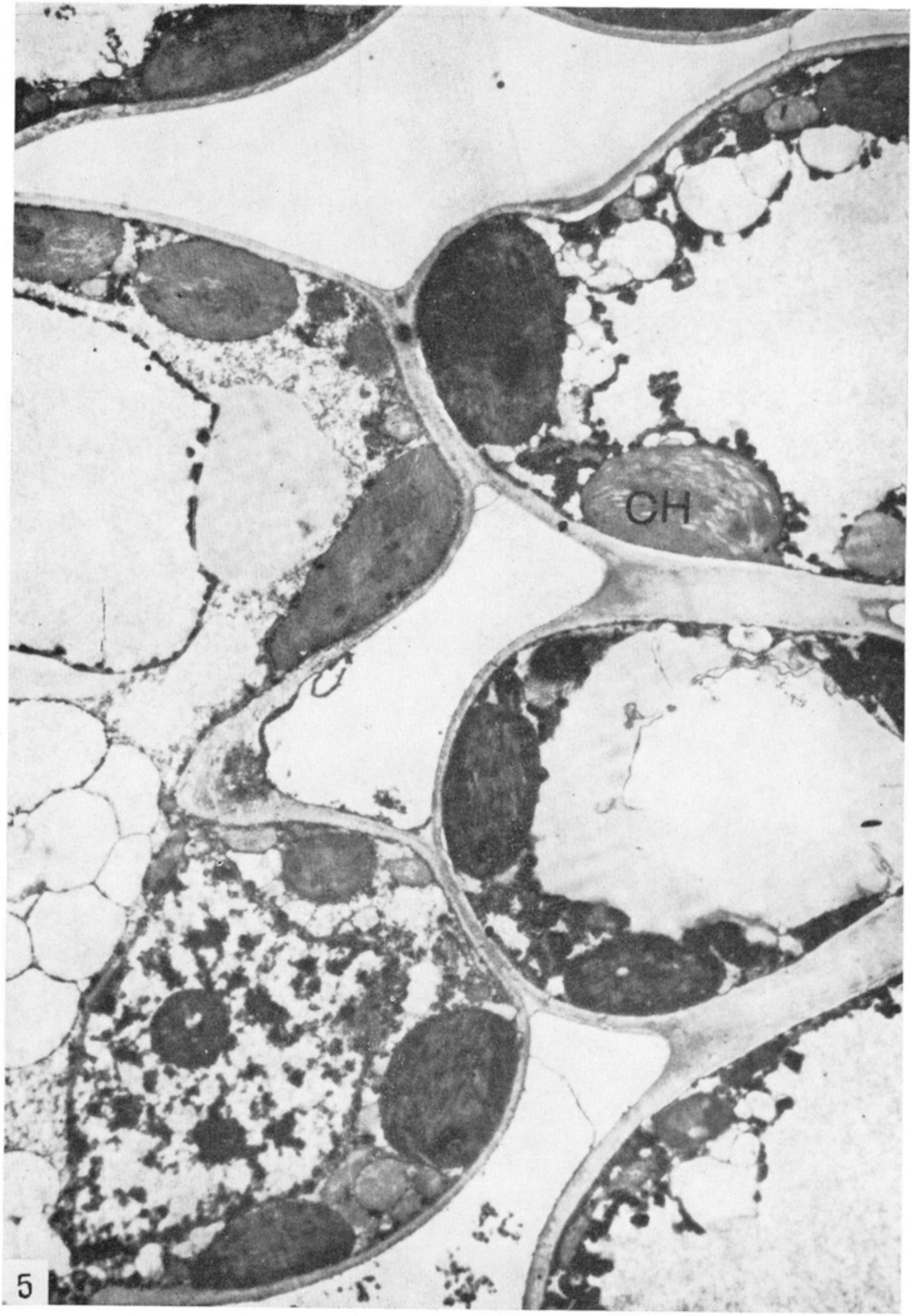
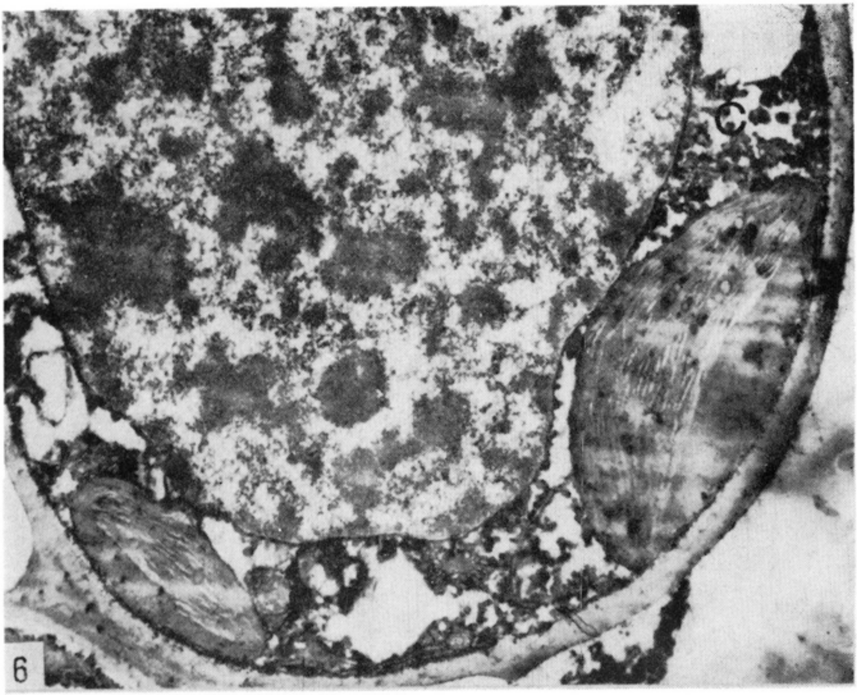


Fig. 5. Section of the mesophyll of initial material obtained from larch susceptible to SO_2. Apart from a slight swelling of thylakoids in some chloroplasts (CH) all other structures are normally developed. ×3 000



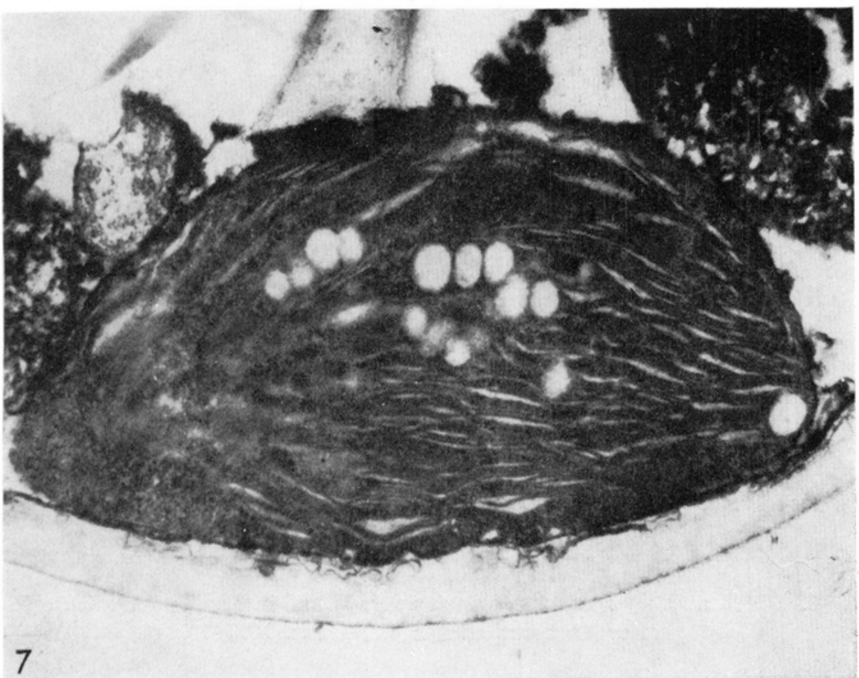
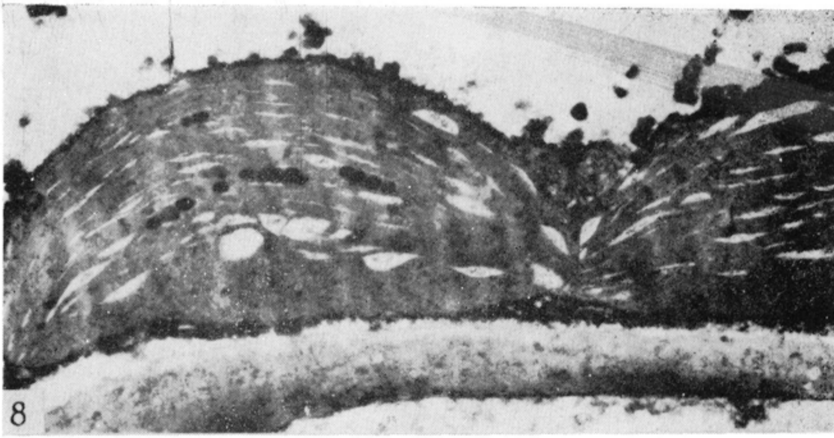


Fig. 6. Part of the mesophyll from larch susceptible to SO_2 after cutting off the shoot and 48 hours in water (control). Granulation of the cytoplasm (C) can be seen. ×5 000

Fig. 7. Chloroplast from the material as in Fig. 6 with significant swelling of thylakoids. ×9 000



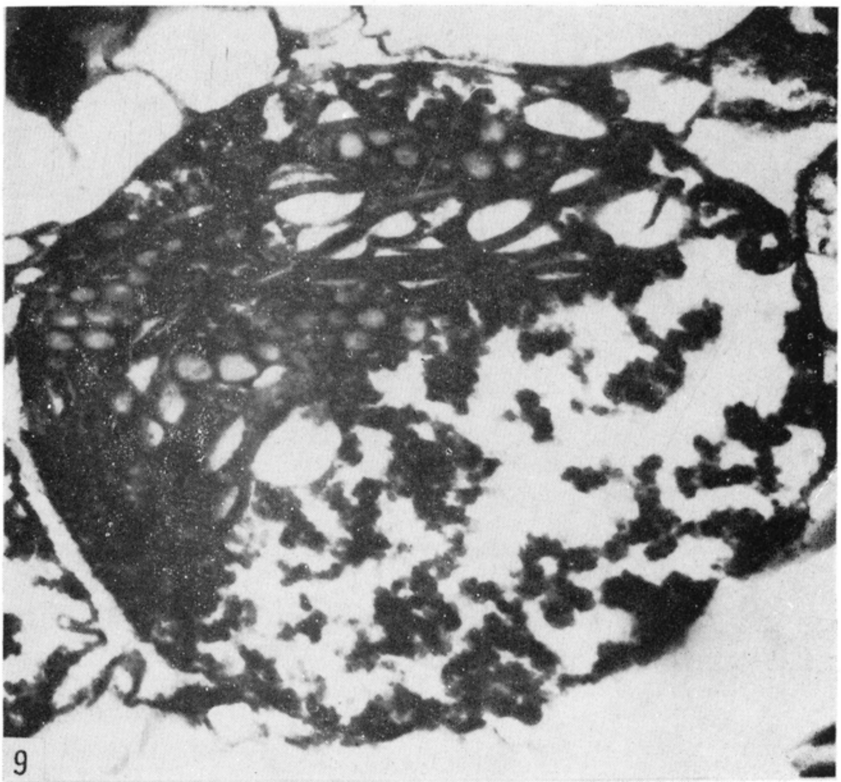


Fig. 8—9. Different stages of chloroplast degradation in larch susceptible to SO_2 after 48 hours of gas treatment. ×7 000

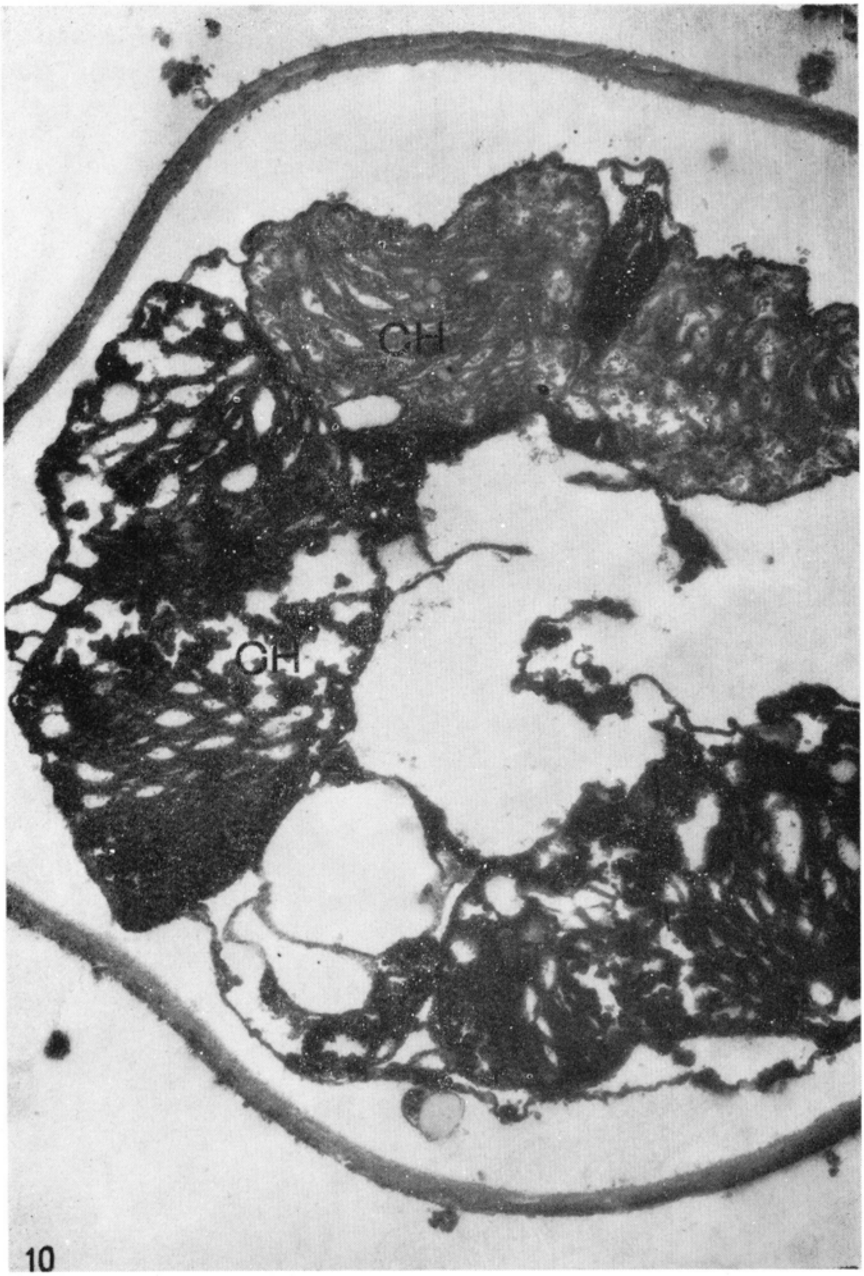


Fig. 10. Strongly plasmolized cell of larch susceptible to SO_2 treatment. Visible changes of the chloroplast (CH) ultrastructure, as well as differences in their degeneration after 48 hours of gas treatment. ×8 000

September 1975). The remaining twigs were kept in similar conditions but without SO_2 treatment — they served as the control. Two days after the last SO_2 treatment fragments of the middle part of the needles were fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer, at 4°C over the night. Next morning this material was washed with the buffer and postfixed with 2% OsO_4 in cacodylate buffer. Pieces of needles were dehydrated with ethanol, acetone, and propylene oxide, and immersed in a mixture of Epon resins. Ultrathin sections were contrasted with uranyl acetate and lead citrate, according to the method by Reynolds (1963). Microphotographs were taken with the use of the microscope JEM 7b.

RESULTS

Experiments were carried out in autumn and although externally the needles did not show any signs of ageing, this process was visible at the level of cell structure, especially as regards the chloroplasts. Numerous plastoglobules were present in the chloroplasts of the initial material obtained from both individuals under study (Fig. 1), and the thylakoids were slightly swollen (Fig. 5). These symptoms, characteristic for plastid degeneration, became slightly more visible after cutting off the shoots and by their placed for 3 days in water (control). Moreover, these changes were more noticeable in the plant susceptible to SO_2 (compare Fig. 2 with Fig. 6, and 7).

Needles treated with SO_2 showed visible signs of degradation after 3 days of the experiment — they became yellow and lost turgor. Yellow colour appeared at the apical side; middle portion of needles (used for studies) had a light green colour. This part contained mesophyll cells at different stages of degradation but in each case the process was much more advanced than in the control needles.

Independently of the degree of resistance to SO_2 treatment degradation of mesophyll cells in both individuals under study may be characterized as follows:

1. Gradual changes of the chloroplast profile, from elipsoidal to oval and spherical (Fig. 8 and 9).

2. Swelling and degradation of thylakoids (Fig. 8 and 9).

3. Granulation of the cytoplasm and of plastid matrix (Fig. 3, 8, and 9).

4. Disintegration of the ribosomes and endoplasmatic reticulum (Fig. 3, 4, and 10).

5. Agglutination of the chromatin (Fig. 3).

6. Cell plasmolysis (Fig. 4 and 10).

Changes induced by SO_2 were present in varying intensity in different parts of the fragments under study, in neighbouring cells, or even in the same cell. Chloroplasts of plasmolized cell presented in Fig. 10 show different symptoms and varying level of degeneration. Degradation of starch in the individual resistant to SO_2 was less advanced (Fig. 3) than in the individual susceptible to SO_2 (Fig. 8 — 10). It seems (compare Fig. 4 and 10) that degradation of chloroplast in the resistant plant occured to a greater extent through the disintegration of thylakoids at preserved matrix, while in the plant susceptible to SO_2 matrix disappeared rather early and thylakoids remained unchanged for a longer period.

DISCUSSION

Larch needles underwent significant changes after 3 day exposure to SO_2. To a certain extent this resulted from the age of the needles since the experiment was carried out in autumn, i.e. just before their natural senescence. It is known that older parts of needles (Malhotra 1976), or leaves developing in worse nutritional conditions (Zahn 1963), are much more susceptible to SO_2.

Fischer et al. (1973) treated leaves of *Vicia faba* with atmospheric SO_2 and found that, similarly as in case of larch, plastids and thylakoids became swollen, and osmophilic granules appeared in the stroma. Also Godzik and Sassen (1974) mentioned that in bean thylakoids disappeared as a result of SO_2 treatement. Moreover, the latter authors observed also some other symptoms which were not noted in case of larch, such as invagination of the internal membrane, appearance of vescicles, vacuoles, and some cristalline inclusions. Ultrastructural changes of the chloroplasts, similar to those noted in larch, such as swelling and disintegration of thylakoids, were also observed in pine needles incubated in aqueous SO_2 — H_2SO_3 (Malhotra 1976). Plasmolysis of cells constitutes one of the symptoms of SO_2 treatement of larch needles. This process was observed also in different material by Wellburn et al. (1972) and Malhotra (1976).

Comparing ultrastructural destructive changes, induced by SO_2 treatement in two larch trees characterized by different susceptibility to this gas, it is well visible that more pronounced changes were noted in the more susceptible individual. It is however known that susceptibility of plants to SO_2 depends on several external factors (Godzik 1976). Furthermore, it was also stated (Fischer et al. 1973) that in the same material it was possible to find cells differing with respect to their reaction to SO_2. Our observations support this statement. Additionally, it was also shown that even in the same cell different stages of plastid degradation were present. Due to the above it is very difficult to compare results obtained not only in different laboratories, but also from individuals in identical experimental conditions.

In larch it is possible to observe two different types of chloroplast degradation: one in which thylakoids disappear first, while matrix remains dense, and the other, characterized by swollen thylakoids and granulated or disappearing matrix. It seems that the first type prevails in plants more resistant to SO_2. It is known that the matrix of chloroplasts contains enzymes of the Calvin cycle. Consequently, the plants were characterized by higher CO_2 uptake and more frequent presence of starch. On the other hand, thylakoids condition the presence of photo systems, necessary for complete photosynthesis. Hence, independently of the fact which of the systems is destroyed, this destruction will lead to the disturbance of photosynthetic activity, although in each case it will refer to its different part. Biochemical and structural studies on chloroplasts treated with SO_2 could contribute many valuable details to this problem.

Acknowledgment

The authors wish to thank Mrs L. Młodzianowska and Mr. P. Karolewski for technical assistence.

REFERENCES

Białobok S., 1976. Studies on the effect of sulphur dioxide and ozone on the respiration and assimilation of trees and shrubs in order to select individuals resistant to the action of these gases. Second Annual Report 1975—1976, Polish Academy of Sciences, Inst. of Dendrology. Kórnik.

Fischer K., 1967. Cytologische und physiologische Wirkungen von Schwefeldioxyd suf höhere Pflanzen. Dissertation Darmschtadt.

Fischer K., D. Kramer, and H. Ziegler, 1973. Elektronenmikroskopische Untersuchungen SO_2 — begaster Blätter von *Vicia faba.* I Beebachtungen an Chloroplasten mit akuter Schädigung. Protoplasma 76: 83—96.

Godzik S. and W. Knabe, 1973. Vergleichende elektronenmikroskopische Untersuchungen der Feinstruktur von Chloroplasten einiger Pinus-Arten aus den Industriegebieten au der Ruhr und in Oberschlesien. Proc. 3rd International Clean Air Congress A: 164—169, Düsseldorf.

Godzik S., and M. M. A. Sassen 1974. Einwirkung von SO_2 auf die Feinstruktur von Chloroplasten von Bohnenblättern. Phytopathol. z. 79: 155—159.

Godzik S., 1976. Pobieranie $^{35}SO_2$ z powietrza i rozmieszczanie ^{35}S u niektórych gatunków drzew. (Uptake of $^{35}SO_2$ from the air and distribution of ^{35}S in some trees). PAN, Wrocław, Zakład Narodowy im. Ossolińskich.

Haselhof E. and G. Lindau, 1903. Die Beschädigung der Vegetation durch Rauch. Berlin: Ed. Gebr. Borntraeger.

Hölte W., 1958. Zur Kenntnis von Wesen und Erscheinungsformen der Schwefelsäurewirkung auf die Pflanzenwelt, Z. J. Pflanzenkrankheiten 65: 32—36.

Inglis F. and P. J. Hill, 1974. The effect of sulphide and fluoride on carbon dioxide uptake by mosses in the light. New Phytol. 73: 1207—1213.

Lorenc-Plucińska G., 1976. The effect of SO_2 on the photosynthesis and respiration of larch in relation to the degree of „resistance" to the action of this gas. In Second Annual Report 1975/1976. Polish Academy of Sciences, Inst. of Dendrology, Kórnik: 33—39.

Lüttke U. and K. Fischer, 1971. Uber die Wirkung von Schwefeldioxyd aus Luftverschmutzungsbestandteil auf physiologische Vorgänge in Pflanzen, Jahrbuch der Technischen Hochschule Darmstadt: 31—41.

Malhotra S. S., 1976. Effects of sulphur dioxide on biochemical activity and ultrastructural organization of pine needle chloroplasts, New Phytol. 76: 239—245.

Puckett K. J., E. Niebver, W. P. Flora and D. H. S. Richardson, 1973. SO_2: its effects on photosynthetic ^{14}C fixation in lichens and suggested mechanism of phototoxicity. New Phytol. 72: 141—152.

Reynolds E. S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208—212.

Ross F. F. and A. J. Clarke, 1973. Sulphur dioxide emissions: an analysis of factors for their effective control., Proc. 3 rd International Clean Air Congress, A: 124—127, Düsseldorf.

Saunders P. J. W. and C. M. Wood, 1973. SO_2 in the environment, its production dispersal, and fate. IN: Air Pollution and Lichens (Ed. by W. Ferry, M. S. Baddley, and D. L. Hawksworth). Athdone Press, London.

Sorauer P. and E. Ramann, 1899. Sogenante unsichtbare Rauchbeschädigungen, Bot. Centralb. 80: 50—251.

Treshow M., 1970. Environment and plant response. McGraw Hill, N. Y.

Wellburn A. R., O. Majernik, and F. A. M. Wellburn, 1972. Effects of SO_2 and NO_2 polluted air upon the ultrastructure of chloroplast, Environ. Pollut. 3: 37.

Zahn R., 1963. Uber den Einfluss verschiedener Umweltfaktoren auf die Pflanzenempfindlichkeit gegenüber Schwefeldioxyd, Z. Pflanzen Kr. Pflanzensch. 70: 81—95.

Ziegler I., 1975. The effect of SO_2 pollution on plant metabolism, Residue Reviews 56: 79—105.

Author's address:
Dr Fortunat Młodzianowski
Institute of Biology, A. Mickiewicz University,
61-713 Poznań, Stalingradzka 14, Poland
Prof. Stefan Białobok
Institute of Dendrology, Polish Academy of Sciences,
Kórnik, Poland

Wpływ dwutlenku siarki na ultrastrukturalną organizację igieł modrzewia

Streszczenie

Pod wpływem SO_2 zaobserwowano szereg zmian w ultrastrukturze mezofilu igieł modrzewia: pęcznienie i degradacja tylakoidów, granulacja cytoplazmy i matriks plastydów, degradacja rybosomów i retikulum endoplazmatycznego, zlepianie chromatyny i plazmoliza komórek.

Porównanie ultrastruktury dwu osobników modrzewia o różnej wrażliwości na SO_2 okazało się trudne, ze względu na brak synchroniczności w reagowaniu poszczególnych komórek na ten gaz.