A Quantitative Evaluation of the Lipid Composition of Leaves of *Aleurites montana* as a Consequence of Growth under 0.3 ppm SO_2 in the Atmosphere

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Gassing during 14 days of *Aleurites* plants with 0.3 ppm SO_2 leads to quantitative modifications of the lipid composition of leaves. The ratio of phospholipids to glycolipids is shifted from 1:3 in control plants to 1:1.8 in *SO_2-plants*. Glycolipids decrease from 62% of total lipids in control plants to 50% in *SO_2-plants*. On the other hand phospholipids increase from 20% in control plants to 28% of total lipids in *SO_2-plants*. This implies that not only the thylakoid membrane but also the mitochondrial membranes and cell membranes of tonoplasts and of the plasmalemma suffer modifications under the influence of SO_2. With respect to the fatty acid composition, despite the drastic change in the lipid composition no alteration in comparison to control plants is seen. Chlorophylls increase in *SO_2-plants* by 24%. Also the content of β-carotene, and of the xanthophylls lutein, violaxanthin and neoxanthin is increased. Only zeaxanthin exhibits a decrease. From the changes in the chlorophyll and carotenoid content it can be concluded that in *SO_2-plants* the light-harvesting-complex (LHCP) is stronger developed (P. He, A. Radunz, K. P. Bader and G. H. Schmid, Z. f. Naturforsch. **51c**, 441–453 and 833–840, 1996).

Introduction

In a previous publication we have reported on the effect of an increased SO_2-content of 0.3 ppm in the atmosphere on growth of the Chinese tung-oil tree *Aleurites montana* (He *et al.*, 1996a). For these experiments 9 months old oil trees were exposed for 14 days to an atmosphere containing 0.3 ppm SO_2 (≈400 μg SO_2/m^3 air). After approx. 6 days 60–70% of the older leaves exhibited chlorosis and were shed. Young expanding leaves, however, apparently adapted to the pollutant and developed to leaves of normal size. In these *SO_2-plants* not only a decrease in the reserve component content like sugars and proteins was observed, but also the biomass forming enzyme ribulose 1.5-bisphosphate carboxylase/oxygenase was decreased. Only the energy equivalent forming CF1-complex exhibited an increase in comparison to control plants. An analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of a photosystem II (PS II) preparation showed that the LHCP was stronger developed in these plants whereas the peptides belonging to the oxygen-evolving complex (OEC), with the molecular masses 33, 24 and 17 kDa occurred in lower concentrations. In the present paper we investigate whether under the influence of 0.3 ppm SO_2 in *Aleurites* plants, which are of great importance for the Chinese lacquer industry (Fang *et al.*, 1981; 1985), in addition to the observed molecular changes in functional protein-enzyme-complexes, also changes in lipids and fatty acids are observed. Under the influence of gassing with 700 ppm CO_2 a quantitative modification of the lipid composi-

Abbreviations: LHCP, light harvesting complex; PS II, photosystem II; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; OEC, oxygen-evolving complex; Monogalactolipid, monogalactosyldiglyceride; Digalactolipid, digalactosyldiglyceride; Sulfolipid, sulfoquinovosyldiglyceride.

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tion and of fatty acids had occurred. Glycolipids as well as phospholipids of these plants are characterized by a higher degree of saturation of fatty acids (He *et al.*, 1996b).

Material and Methods

Cultivation of plants

Aleurites montana plants were grown in an fully climatized growth chamber with a light/dark cycle of 14/10 hours, a day temperature of 26 °C, a night temperature of 22 °C and a relative humidity of 60%. For the SO_2-gassing 9 months old *Aleurites* plants were kept in glass compartments in the same growth chamber for 14 days at 0.3 ppm SO_2. Light, temperature and humidity conditions corresponded exactly to those of the control plants. Leaves for lipid analyses were harvested after 14 days of SO_2-gassing and lyophylized.

Lipid analyses

Extraction of lipids, their qualitative and quantitative analysis as well as analyses by gas chromatography of fatty acids are described in previous publications (He *et al.*, 1996b; Radunz 1966a, b, 1972, 1976).

Table I. Composition of purified ether soluble lipids of *Aleurites montana* cultivated under normal atmospheric conditions (control plants) and under 0.3 ppm SO_2 in the atmosphere (*SO_2-plants*). *SO_2-plants* were exposed at the age of 9 months for 14 days to SO_2. Determined lipids were calculated in percent of total lipids (100%). Not determined are tocopheroles, plastoquinones and waxes.

Lipids	*SO_2-plants*	Control plants
Monogalactosyldiglyceride	28.5	32.3
Digalactosyldiglyceride	16.4	24.8
Sulfoquinovosyldiglyceride	2.7	3.2
Sterylglycoside	1.9	1.2
Cardiolipin	1.8	1.0
Phosphatidylethanolamine	3.6	3.6
Phosphatidylglycerol	5.9	3.8
Phosphatidylcholine	11.2	9.1
Phosphatidylinositol	5.0	2.5
Chlorophyll *a*	13.5	11.1
Chlorophyll *b*	4.6	3.6
Chlorophyll *a/b*	2.89	3.06
β-Carotene	1.35	1.11
Lutein	2.56	1.93
Zeaxanthin	0.14	0.35
Violaxanthin	0.33	0.19
Neoxanthin	0.52	0.22
Phospholipids/glycolipids	1/1.8	1/3.0
Carotenoids/chlorophyll	1/3.8	1/3.9

Values represent averages of 10 determinations and deviate by ± 3%.

Results and Discussion

Influence of SO_2 on the Lipid Composition

In order to analyze the influence of 0.3 ppm SO_2-content in the atmosphere on lipids and fatty acids of leaves of *Aleurites*, leaves of 9 months old plants that had been cultivated for 14 days under SO_2 were harvested, lyophilized and the lipids extracted with methanol, acetone and diethylether. With respect to the control plants it should be noted that, as described already in a preceding publication (He *et al.*, 1996a), under the effect of SO_2-gassing after approx. 6 days 60–70% of the older leaves exhibited chlorosis (Elstner and Hippeli, 1995) and were shed, whereas younger expanding leaves adapted to the SO_2-conditions and developed to leaves of normal size. This means that preparations of *SO_2-plants* represent younger material than preparations from control plants. Therefore, the control preparations might have a fatty acid composition with a higher saturation degree, since in dependence on the age of leaves the content of saturated fatty acids increases (Radunz, 1966b). The portion of ether soluble purified lipids comprises in SO_2 11.1% and in control plants 9.9% of leaf dry material. This means that *SO_2-plants* correspond with respect to their lipid content to any other higher plants and to *CO_2* plants (Radunz, 1966 a; He *et al.*, 1996b). The composition of the lipids, however, shows with *SO_2-plants* stronger differences than with *CO_2-plants* (He *et al.*, 1996b) (Table I). Whereas in control plants glycolipids make up for 62% of total lipids, glycolipids make up for only 50% in *SO_2-plants*. On the other hand, phospholipids increase from 20% of total lipids in control plants to 28% of total lipids in *SO_2-plants*. This represents a decrease of glycolipids by 19% and an increase of phospholipids by 38% under the effect of SO_2-gassing. Among glycolipids it is digalactolipid and among the phospholipids it is phosphatidylintositol which is subject to the largest modification. This strong decomposition of glycolipids and the strong increase in phospholipids hints at the modification of the thylakoid membrane, the mitochondrial membrane as well as of that of all other cell membranes such as tonoplasts and the plasmalemma. It should be borne in mind that glycolipids are the main components of thylakoids and phospholipids are the main components of mitochondrial and of cell membranes (Frentzen and Heinz, 1983; Quinn and Williams, 1983; Radunz, 1972, 1976). A marked difference is also seen in the chlorophyll *a* and *b* composition and in the ratio of these two chlorophylls. Whereas in control plants the chlorophyll content comprises 15% of total lipids and the *a*:*b* ratio is 3.06:1, the chlorophyll content of *SO_2-plants* increases by 18% and the ratio a:b shifts to 2.89:1. A similarly drastic increase in *SO_2-plants* is seen with the carotenoids. Nevertheless, the ratio carotenoids to chlorophylls remains constant under the two culture conditions. The increase in carotenoids and chlorophylls as well as the decrease of the chlorophyll *a*/*b* ratio in *SO_2-plants* suggests that the light-harvesting-complex is stronger developed in *SO_2-plants*. This observation fits into results obtained by the analysis of chloroplast peptides with SDS-PAGE (He *et al.*, 1996a and b) and apparently represents a protective measure of *Aleurites* plants to SO_2-stress.

Table II. Comparison of the fatty acid composition of leaf lipids of *Aleurites montana* grown under normal conditions (Control plants) and a SO_2 content of 0.3 ppm in the atmosphere (*SO_2-plants*). *SO_2-plants* were grown at the age of 9 months for 14 days under 0.3 ppm SO_2 in the atmosphere. Fatty acids are calculated in percent of total fatty acids (100%).

Fatty acids	*SO_2-plants*	Control plants
$C_{12:0}$	0.2	0.2
$C_{14:0}$	1.0	1.4
$C_{16:0}$	11.9	12.8
$C_{16:1\ cis}$	0.5	0.6
$C_{16:1\ trans}$	1.0	1.2
$C_{18:0}$	2.0	2.1
$C_{18:1}$	2.2	2.0
$C_{18:2}$	8.4	10.2
$C_{18:3}$	72.8	69.5
Saturated/unsaturated fatty acids	0.18	0.19
C_{16}/C_{18}-fatty acids	0.16	0.17

Values represent averages of 10 individual determinations and deviated by ± 3%.

Influence of SO_2 on the Fatty Acid Composition

Concerning the fatty acid composition SO_2-gassed plants and control plants show no significant differences. As shown in Table II, the saturation degree as well as the chain length is the same in the two types of plants. Slight differences are only seen in the portion of linoleic and linolenic acids. Whereas linolenic acid makes up for 69% of total fatty acids in control plants, the content increases in *SO_2-plants* to 73%. On the other hand linoleic acid which in control plants makes up for 10% goes down to 8% in *SO_2-plants*. All together this are only minor differences.

From the drastic shift of the lipid composition represented by the decrease of glycolipids and the increase of phospholipids in *SO_2-plants*, an increase in the content of saturated fatty acids was expected in these plants as in glycolipids unsaturated fatty acids make up for approx. 90% of total fatty acids, as described earlier (Radunz 1972, 1976, Quinn and Williams, 1983). As, however, the saturation degree of fatty acids in *SO_2-plants* in comparison to control plants remains unchanged, these results show, that the degree of unsaturated fatty acids has increased in the *SO_2-plants* either in glycolipids or phospholipids or in both lipid classes. It looks as if the decrease of the fluidity of the thylakoid membrane induced by the reduction of glycolipids is compensated by an increase of unsaturated fatty acids.

The Chinese Tung Oil Tree *Aleurites montana* occurs in a highly SO_2-polluted area. The regions of the Central South of China, e.g. Hunan are characterized by perennial high SO_2-values in the air which lie between 0.5–1.15 mg SO_2/m^3 air. This high SO_2-content in the air is produced by local industries (e.g. brick kilns, aluminum production, etc.) and private heating, both using a particularly sulphur-rich coal as energy source. This situation affects growth of many plants and an example being the Tung Oil Tree of the present studies. The value of 0.3 ppm SO_2 used in the present study lies slightly below the range given above and would correspond to approx. 400 μg SO_2/m^3 air which is nevertheless 5 times the limit value of 80 μg SO_2/m^3 air beyond which smog alarm is given in Northrhine-Westfalia/Germany.

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