Influence of CO_2 and SO_2 on Growth and Structure of Photosystem II of the Chinese Tung-Oil Tree *Aleurites montana*

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Three months old plants of the Chinese tung-oil tree *Aleurites montana* were cultivated for 4 months in air containing an increased amount of 700 ppm CO_2. During the exposure to 700 ppm CO_2 the plants exhibited a considerably stronger growth (30–40%) in comparison to the control plants (grown in normal air). In these CO_2-plants during the entire analyzing period the amount of soluble proteins, of soluble sugars and the chlorophyll content were lower than in control plants. The protein content, referred to leaf area, increased during this time in both plant types by approx. 50% but with a different time course. The increase is faster in CO_2-plants compared to control plants, and ends up with similar values in both plants after 4 months. No difference is seen between sun and shade leaves.

The chlorophyll content in both sun and shade leaves is 20% lower in CO_2-plants. Whereas the chlorophyll content in sun leaves stays constant during development, it has increased in shade leaves by 20% at the end of the 4 months period. The content of soluble sugars is lower in CO_2-plants compared to control plants. The difference is bigger in sun leaves than in shade leaves.

The ribulose 1.5-bisphosphate carboxylase/oxygenase content almost doubles within the experimentation period, but seems to be subject to large variations. CO_2-plants contain in general less ribulose 1.5-bisphosphate carboxylase/oxygenase than control plants. The content of coupling factor of photophosphorylation is 20% lower in CO_2-plants when compared to control plants and remains during development more constant in CO_2-plants. The molecular structure of the photosystem II-complex undergoes under the influence of the increased CO_2-content a quantitative modification. The light harvesting complex (LHCP) and the extrinsic peptide with the molecular mass of 33 kDa increase in CO_2-plants.

Gassing with SO_2 (0.3 ppm in air) leads to a strong damage of the plants. The damaging influence is already seen after 6 days and leads to a partial leaf-shedding of the tree. In the visually still intact remaining leaves the chlorophyll content referred to unit leaf area decreases by 63%, that of soluble sugars by 65%, the content of soluble proteins and that of Rubisco decrease by 26% and 36% respectively. The light harvesting complex and the chlorophyll-binding peptides (43 and 47 kDa) increase whereas the extrinsic peptides decrease. It looks as if the simultaneous application of SO_2 (0.3 ppm) and increased CO_2 (700 ppm) releaves the damaging effect of SO_2. Plant growth does not exhibit a difference in comparison to control plants. Soluble proteins and chlorophyll increase by 27% and 33% and the ribulose 1.5-bisphosphate carboxylase/oxygenase content as well as that of soluble sugars increases by 18 respectively 14%. The peptide composition of photosystem II shows a quantitative modification. The LHCP increases and the chlorophyll-binding peptides and the peptides with a molecular mass smaller than 24 kDa are reduced. The quantity of extrinsic peptides appears unchanged.

Ribulose 1,5-bisphosphate carboxylase/oxygenase and the CF_1-complex of *Aleurites* are immunochemically only partially identical to the corresponding enzymes of *Nicotiana tabacum* as demonstrated by tandem-cross-immune electrophoresis.

Introduction

The CO_2-content of air as well as the content of gas pollutants such as SO_2 are constantly increasing (Houghton *et al.*, 1990). Not too much is known on long-term effects of these gases on growth of higher plants. Experiments exist which describe the so-called short-term effect of an increased CO_2-content (Makino, 1994; Stitt, 1991; Sage *et al.*, 1989; Yelle *et al.*, 1989; Robertson and Leech, 1995). These reports deal with structural alterations of chloroplasts (Robertson and Leech, 1995; Cave *et al.*, 1981), with the effect on photosynthetic electron transport (Sage *et al.*, 1989; Makino, 1994; Lawlor and Mitchell, 1991; Stitt, 1991, Stitt and Schulze, 1994), with modifications of the photosystem I- and II-complexes and quantitative changes concerning the polypeptides of these functional complexes (Robertson and Leech, 1995,

Sage *et al.*, 1989; Makewicz *et al.*, 1995 b, c; Radunz and Schmid, in preparation). In the present study the influence of the two gases CO_2 and SO_2 on growth of the Chinese Tung-Oil Tree (*Aleurites montana*) is followed during an entire vegetation period. *Aleurites montana* belongs to the genus *Euphorbiaceae*. In China its seeds represent an important crop yield of about 4×10^5 tons of oil per year (Fang *et al.*, 1985; Fang and Que, 1981). The oil is, due to its content of $60-80\%$ C_{18}-trien fatty acids with Δ^9 *cis*, Δ^{11} *trans*, Δ^{13} *trans* configuration, a highly estimated raw material in the laquer industry (Fang *et al.*, 1985; Gunstone *et al.*, 1994). Air pollution, amongst others by SO_2, increasingly endangers this crop.

In our studies we have grown small trees in a fully climatized growth chamber for 3 months and then continued cultivation by exposing the plants

Fig. 1. 7 months old plants of *Aleurites montana*. Left: control plant grown in normal air with 350 ppm CO_2; right: *CO_2-plant* grown after the age of 3 months for 4 months in air containing 700 ppm CO_2.

in the same growth chamber to 700 ppm CO_2. The first analysis of chlorophylls, proteins, soluble sugars of the enzymes Rubisco and coupling factor of photophosphorylation (CF_1) as well as measurements of photorespiration (He *et al.*, in preparation) were carried out after 2 months of cultivation in 700 ppm CO_2. Thereafter these analyses were carried out twice in monthly intervals which means that the plants in the last stage were 7 months old. For a control, a number of plants were kept under normal CO_2 (350 ppm) in the same growth chamber. After 4 months, the influence of the increased CO_2-content on the peptide composition of chloroplasts and of the photosystem II-complex was analyzed. After this experimental period, a number of control plants were exposed for two further weeks to a gas atmosphere which contained in addition 0.3 ppm SO_2. Thereafter, the plants were exposed to the combination of 0.3 ppm SO_2 and an increased content of 700 ppm CO_2. The results concerning this study are reported in the present paper. The influence of the increased CO_2-content on the lipid, the fatty acids and carotenoid metabolism as well as on the photorespiratory behaviour will be published in a separate paper (He *et al.*, in preparation).

Material and Methods

1. Cultivation of Aleurites-plants

Aleurites montana plants were cultivated in a fully climatized growth chamber in a light/dark cycle of 14 h/10 h at a day temperature of 27°C, night temperature of 22° and 60% relative humidity. Seeds came from the Central South Forestry University, Zhuzhou, Hunan. Voucher specimens of *Aleurites montana* and *Aleurites fordii* are available in the laboratories of Bielefeld. Cultivation of plants under increased CO_2- and/or SO_2-content was carried out in the same growth chamber in different glass compartments. In the glass compartments the plants were exposed to the same conditions as in the uncompartmented growth chamber. CO_2-plants were grown at 700 ppm CO_2 in the gas phase and SO_2-plants with 0.3 ppm SO_2. Dosage was achieved with a peristaltic pump (Perimax 12) from Spetec GmbH, 85435 Erdingen and a suitable valve system (Schmid *et al.*, 1981; Ishii and Schmid, 1982).

2. Isolation of chloroplasts and preparation of photosystem II-particles

Chloroplasts were isolated following the methods described by Oku and Tomita (1976) and He (1987) by fractioning centrifugation using Tricine buffer, pH 7.4, (buffer composition: 50 mM Tricine, 0.4 M sucrose, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM EDTA und 0.6% polyethyleneglycol 4000). The chloroplast sediment was suspended in the above buffer containing in addition 2% Triton and sonicated for 3 x 20 seconds and than shaken for 30 minutes. Thereafter, the suspension was centrifuged for 30 sec at 15,000 x *g* with the supernatant used for determination of the CF_1-complex by rocket-immune electrophoresis (Radunz and Schmid, 1989; Schmid *et al.*, 1993).

Isolation of PS II-particle preparations was carried out according to Berthold *et al.* (1981).

3. "Leaf Preparations"

A defined amount of *Aleurites montana* leaves was cut into small pieces and homogenized in the presence of 0.03 M Tris-buffer, pH 8.0, containing 4 mM $MgCl_2$, 1 mM EDTA and 2% Triton X-100 by means of a stick blender (Ystral GmbH, Typ 1020). The homogenate was filtered through 8 layers of cheese cloth. The filtrate was 3 times sonicated for 20 sec, stirred for 30 min at room temperature and centrifuged for 30 min at 15,000 x *g*. The supernatant was used for the determination of Rubisco in the rocket-immune electrophoresis according to Schmid *et al.* (1993).

4. SDS-Gel electrophoresis and Western Blot

Peptides of chloroplasts and of the PS II-particle preparations were analyzed at 30 mA at room temperature in SDS-PAGE using a 1.5 mm gel consisting of a 10–20% gradient gel and a 3% collecting gel (Laemmli, 1970; Weber and Osborn, 1969). The gel was stained with Coomassie Brilliant Blue. For the electrophoresis 20 µl of the individual peptide fractions (corresponding to 10–20 µg chlorophyll) were incubated for 60 min at 50°C with 20 µl of sample buffer (composition of the sample buffer: 1 M Tris, pH 6.8, containing 10% glycerol, 2% SDS, 0.01% bromophenol blue and 0.1 M dithiothreitol).

For Western blotting the separated peptides were transferred in lateral direction to nitrocellulose membranes by 20 hrs diffusion at room temperature (Renart *et al.*, 1979). Blocking of membranes was achieved with 2.5% fish gelatine. The dilution of the protein used and that of the lipid antisera is given in the legends of the respective figures. The second antibody, a peroxide-conjugated pig-immunoglobulin to rabbit immunoglobulin (Anti-rabbit IgG, DAKO) was diluted 1:500. Labelling of the specifically bound antibodies was obtained by the reaction of the peroxidase with H_2O_2 and 4-chloro-1-naphthol (Sigma, Munich).

5. Chlorophyll-, protein- and sugar-determinations

Chlorophyll was photometrically determined according to Schmid (1971) in 90% aqueous methanol. The protein determination was made according to Smith *et al.* (1985). For the determination of the sugar content the samples were incubated for 15 min at 20°C with 1 ml 5% phenol solution and 4 ml H_2SO_4, centrifuged and photometrically measured at 485 nm (Roughan and Batt, 1968). The determined sugars were calculated as galactose. Determinations of chlorophyll, proteins and sugars were carried out in series of at least 10 individual determinations.

6. Immunological determinations

The immunological test reactions of immuno double diffusion, of tandem-cross immune electrophoresis for comparative protein analyses, and of rocket immune electrophoresis for the quantitative determination of proteins and enzymes were carried out in 1% agarose gel. The double immuno diffusion was carried out in 0.06 M Na_2HPO_4/ KH_2PO_4, pH 7.4, and the tandem-cross and rocket immune electrophoreses were carried out in Tris-barbital buffer, pH 8.6, (buffer composition: 73 mM Tris-(-hydroxymethyl)aminomethane, 250 mM 5.5-diethylbarbituric acid and 0.34 mM calcium lactate (Nespoulous *et al.*, 1988; Radunz and Schmid, 1988; Schmid *et al.*, 1993). The used protein, lipid and carotenoid antisera were characterized and described in earlier papers (Lehmann-Kirk *et al.*, 1979 a, b; Makewicz *et al.*, 1994, 1995 a, c; Radunz, 1971, 1972, 1976, 1984; Radunz and Berzborn, 1970; Radunz and Schmid, 1973, 1975;

Radunz and Bader, 1982; Radunz *et al.*, 1984 a, b; Kruse *et al.*, 1993; Schmid *et al.*, 1993).

Results

Influence of 700 ppm CO_2

In order to study the influence of an increased CO_2-content of the atmosphere on growth of *Aleurites montana*, 3 months old plants of the Chinese Tung-Oil Tree *Aleurites montana* were grown over a time period of 4 months in air containing 700 ppm CO_2. For a comparison plants were kept in normal air (i.e. 350 ppm CO_2) over the same time period under otherwise identical conditions. All plants were cultivated in glass compartments in the same growth chamber at identical light, temperature and relative humidity conditions. This time period of 4 months was chosen to analyze besides the so-called short-term effect of CO_2 (Makino, 1994) also a long-term effect on plant growth. During this period in three intervals namely after 2, 3 and 4 months the chorophyll-, protein- and sugar-content of the leaves were analyzed.

At the same time the peptide composition of photosystem II and that of chloroplasts was analyzed. By means of immunological methods namely by rocket immune electrophoresis the concentration of the bifunctional enzyme Rubisco as well as that of the coupling factor of photophosphorylation (CF_1) was determined using monospecific antisera. We tried to distinguish between sun and shade leaves, since chloroplasts differ with respect to their morphological structure and their physiological efficiency in dependence on the light intensity to which leaves are exposed (Boardman, 1977; Anderson, 1986; Malkin and Fork, 1981; Melis, 1984, Rühle and Wild, 1985; Anderson *et al.*, 1988). After 7 months of experimentation time the PS II-complex was isolated and the peptide compostion analyzed by SDS-PAGE. With monospecific antisera to carotenoids and lipids the quality and quantitiy of lipids and carotenoids bound to these peptides was determined.

The comparative results of CO_2-plants (700 ppm CO_2 in air) and control plants (350 ppm CO_2 in air) with respect to protein-, chlorophyll-, Rubisco- and CF_1-content are given in Table I. Values are referred to leaf area and protein. Moreover, distinction is made between sun and shade leaves

Tab. I. Protein-, chlorophyll-, sugar-, ribulose 1.5-bisphosphate carboxylase/oxygenase (Rubisco)- and content of coupling factor of photophosphorylation (CF_1) in sun and shade leaves of the Chinese Tung-Oil Tree *Aleurites montana* cultivated in air containing 700 ppm CO_2 in comparison to plants grown under normal air conditions.

Aleurites montana		Age of plants in months/ duration of CO_2- gassing in months	Protein/ area [mg/cm^2]	Rubisco/ area [mg/cm^2]	Chlorophyll/ area [mg/cm^2]	Chloro- phyll *a/b*	Sugar/ area [mg/cm^2]	Rubisco % of protein	CF_1 % of protein	Chloro- phyll % of protein
Sun leaves	control plants	5/0	0.94	0.26	0.043	3.00	0.19	27.3	6.6	4.6
	CO_2-plants	5/2	0.81	0.26	0.035	3.06	0ö.10	32.5	5.1	4.4
Shade leaves	control plants	5/0	0.82	0.15	0.046	2.95	0.15	18.7	–	4.3
	CO_2-plants	5/2	1.18	0.31	0.037	3.13	0.18	26.1	–	4.1
Sun leaves	control plants	6/0	1.57	0.45	0.038	2.70	0.61	28.9	5.9	2.4
	CO_2-plants	6/3	0.79	0.22	0.032	2.91	0.17	27.3	4.7	4.0
Shade leaves	control plants	6/0	2.39	0.50	0.048	2.48	0.25	20.7	–	2.0
	CO_2-plants	6/3	0.90	0.24	0.036	2.67	0.25	26.5	–	4.1
Sun leaves	control plants	7/0	1.47	0.79	0.041	3.12	0.22	53.5	4.0	2.8
	CO_2-plants	7/4	1.55	0.45	0.040	2.95	0.19	29.0	3.7	2.6
Shade leaves	control plants	7/0	1.33	0.57	0.055	2.82	0.24	42.6	–	4.2
	CO_2-plants	7/4	1.31	0.53	0.046	3.26	0.17	40.1	–	3.5

3 months old plants were taken and further cultivated in a gas atmosphere containing 700 ppm CO_2 in air. The first analyses with the CO_2-plants were carried out after two months of cultivation in the high CO_2-concentration. From there onward the next analyses were carried out in monthly intervals. The maximal duration of cultivation in 700 ppm CO_2 was 4 months. Protein and chlorophyll values deviated by 2 to 3%.

(Table I). It is observed that if the chlorophyll *a/b* content is referred to leaf area, *Aleurites* shade leaves have a higher chlorophyll content than sun leaves. This corresponds to what is known from the literature (Boardman, 1977; Anderson, 1986; Malkin and Fork, 1981; Melis, 1984, Rühle and Wild, 1985; Anderson *et al.*, 1988). Plants which have been grown under the increased CO_2 content of 700 ppm have, – and this is valid for sun as well as for shade leaves-, a lower chlorophyll content in comparison to the controls. Also in CO_2-plants the chlorophyll *a/b* ratio seems to be higher than in control plants. If the chlorophyll content is referred to protein then the chlorophyll content in CO_2-plants is also lower in comparison to the control plants. Here, with this reference it appears that the chlorophyll content of shade leaves is lower than that of sun leaves. As chlorophyll *b* occurs exclusively in the light-harvesting complex, this observation together with the fact that the chlorophyll content is generally lower in CO_2-plants, should mean that in plants grown under high CO_2 the light harvesting complex has undergone a reduction. This, however, is not confirmed by the peptide analysis of the photosystem II complex (Fig. 4).

Whereas the chlorophyll content stays relatively constant during the 7 months test period, the protein content increases per leaf area during this time. This increase represents in sun as well as in shade leaves 55–60% and is apparently due to an increase in Rubisco. The bifunctional enzyme makes up for 1/3 of all soluble proteins in young leaves and approx. 1/2 of all soluble proteins in older leaves. Under the influence of the increased CO_2-content young leaves exhibit a reduced protein content. In the course of aging, however, after approx. 6 months an equalization of the protein content in CO_2- and control plants is observed.

More clear than the protein values are the values for soluble sugars. The sugar content appears lower in plants grown at the high CO_2-concentration than in control plants. Differences in the sugar content appear better equilibrated or more constant with shade leaves of CO_2-plants than in sun leaves. Possibly in shade leaves the sugar decomposition is lower.

A quantitative immunological determination of the CF_1-complex and Rubisco by means of rocket immune electrophoresis led to the results shown in Table I. The Rubisco content increases in *Aleurites* during the 3 months period two-fold. This increase is observed regardless whether Rubisco is referred to total protein or to leaf area. A comparison of the Rubisco content in CO_2-plants and control plants shows that a difference is only seen after a growth period of 4–5 months. Then, the Rubisco concentration appears to be higher in control plants than in CO_2-plants.
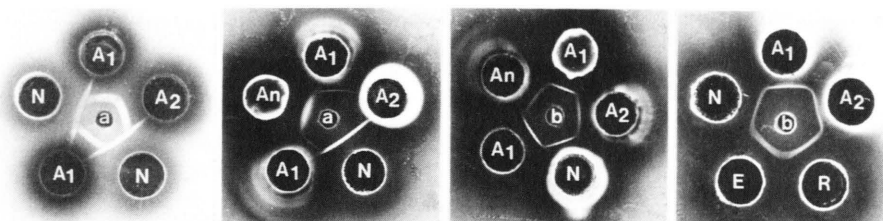
Fig. 2. Demonstration of partial immunochemical identity between ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and coupling factor of photophosphorylation (CF_1) from *Aleurites montana, N. tabacum, Rhizinus communis* and *Euphorbia cyparissias* by double immuno diffusion according to Ouchterlony. a. antiserum to Rubisco; b. antiserum to CF_1. As an antigen leaf preparations were used: A_1, *Aleurites montana* "control plants"; A_2, *Aleurites montana* CO_2-plants; N, *N. tabacum* Su/su; A_n, *Antirrhinum majus*; R, *Rhizinus communis*; E, *Euphorbia cyparissias*.

Concerning the coupling factor of photophosphorylation (CF_1) we were able to show that the ATP-synthase referred to protein decreases during the test period. As, however, the protein content increases during this test period in *Aleurites* plants, the CF_1 content seems to stay more or less constant. CO_2-plants seem to have, as measured during the 3 months period, a lower CF_1-content. As in this case no difference between sun and shade leaves was made, the higher CF_1-content of control plants cannot be correlated with a reduced grana structure of CO_2-plants (Radunz and Schmid, 1989).

Comparative immunological studies of Rubisco and CF_1 in the double immuno diffusion test have shown that the enzymes from *Aleurites* are not fully identical to those of *N. tabacum* and *Antirrhinum majus* (Fig. 2). The enzymes are immunologically only partially identical, as seen by unilateral spurs (Fig. 2) in the immuno double diffusion test. We were able to show, that the CF_1 enzyme complex from *Aleurites* is only partially identical to

other representatives of *Euphorbiaceae* such as *Rhizinus communis* or *Euphorbia cyparissias*. It should be noted that the CF_1-complex is serologically identical between the two *Euphorbiaceae* representatives. The immunological differences are clearly seen by tandem-cross immune electrophoresis (Fig. 3).

After 7 months PS II-particles were isolated from the CO_2-plants and control plants according to the procedure of Berthold *et al.* (1981). In Table II the chlorophyll and protein values of PS II-preparations were compared with those of the chloroplasts. The PS II-complex consists, as seen by SDS-PAGE and as known for other higher plants, of the light harvesting peptides with the molecular masses 28, 26, 25 and 24 kDa, the chlorophyll-binding peptides from the proximal antenna with the masses 43 and 47 kDa and the core peptides D1/D2. Also with *Aleurites* these core peptides not only occur in the monomer form but also in a heterodimer form (Fig. 4). Moreover, electrophoresis shows the 3 extrinsic peptides of
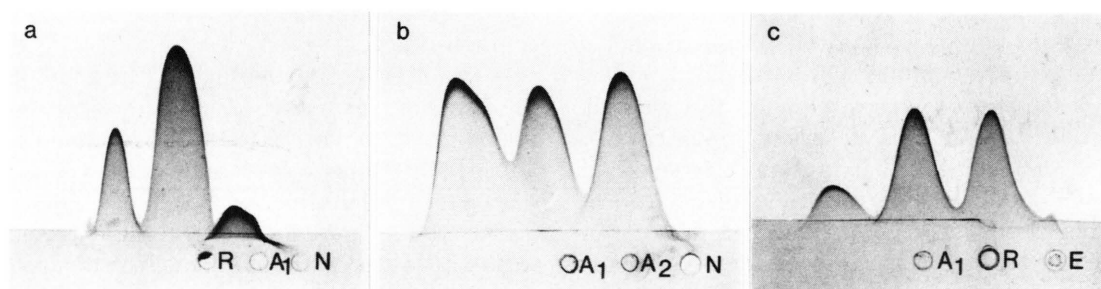
Fig. 3. Demonstration of partial immunochemical identity between ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and coupling factor of photophosphorylation (CF_1) of *Aleurites montana, N. tabacum* Su/su, *Rhizinus communis* and *Euphorbia cyparissias* by tandem-cross immune electrophoresis. a. 1% antiserum to Rubisco in the gel; b. and c. 1.5% antiserum to CF_1 in the gel. As antigen leaf preparations were used: N of *N. tabacum* Su/su; A_1 of *Aleurites* control plants; A_2 of *Aleurites* CO_2-plants; R of *Rhizinus communis*; E of *Euphorbia cyparissias*.

Table. II. Chlorophyll/protein ratio in chloroplasts and photosystem II-preparations of *Aleurites montana* depending on different CO_2-contents in the atmosphere and depending on the age of the plants. 3 months old plants were cultivated for 4 months under 700 ppm CO_2.

Aleurites montana	Age of plants/months duration of CO_2-gassing	Ratio chlorophyll/protein	Chlorophyll a/b
Chloroplast from			
Control plants	7/0	1/16.3	3.02
CO₂-plants	7/4	1/22.5	2.89
PS II-preparations from			
Control plants	7/0	1/4.2	2.55
CO₂-plants	7/4	1/4.6	2.45

the oxygen evolving complex with the molecular masses of 33, 21 and 16 kDa. Only quantitative differences are seen in the peptide composition of PS II-particles from *Nicotiana tabacum* and *Aleurites*. It looks as if the smallest extrinsic peptide and the two chlorophyll-binding peptides occur in higher concentrations in *N. tabacum*. In the *Aleurites* preparations, apparently, due to differences in the response to the isolation procedure, strong impurities of the α- and γ-subunit of the coupling factor of photophosphorylation (molecular masses 37 and 56 kDa) are seen.

Four months of cultivation of *Aleurites* under increased CO_2-concentration (700 ppm) lead to a quantitative modification of the peptide composition of photosystem II. As seen from comparative SDS-PAGE analyses (Fig. 4), the light harvesting complex is stronger developped in *C0₂*-plants than in control plants. In contrast to this, control plants have more of the chlorophyll-binding peptides (i.e. the proximal antenna) and of the 33 kDa extrinsic peptide.

Influence of SO₂

In order to analyze the influence of the air pollutant SO_2 on growth of *Aleurites*, 9 months old plants were kept for 14 days in an atmosphere supplemented with 0.3 ppm SO_2. The other external factors such as light, temperature and humidity corresponded to those of the control plants. After approx. 6 days large leaf areas turned brown and approx. 8 days later a strong leaf-shedding occurred affecting 60–70% of the leaves. Analysis of chlorophylls, proteins and soluble sugars as well as that of Rubisco and CF_1 showed, as demonstrated in Table III, that these plants had undergone a drastic stress situation. Referred to protein, chlorophyll a/b decreases by 50% and soluble sugars by 52%. Rubisco suffers a 14% reduction. Only the CF_1-complex occurs in *S0₂*-plants in higher concentrations. As the protein content decreases during this time, the CF_1-content merely seems to stay constant during this development.
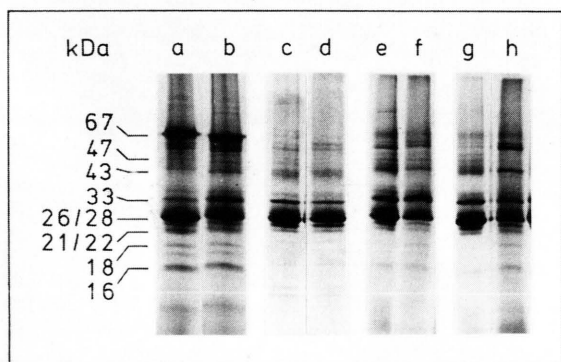
Fig. 4. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the peptide composition of chloroplasts and photosystem II-preparations of *Aleurites montana* plants grown under different air conditions in a. SDS-PAGE with chloroplasts from *CO₂*-plants; b. SDS-PAGE with chloroplasts from control plants; c. SDS-PAGE with PS II particles from *CO₂*-plants; e. SDS-PAGE with PS II particles from *SO₂*-plants; g. SDS-PAGE with PS II particles from *SO₂ -/CO₂*-plants; d., f. and h. SDS-PAGE with PS II-particles from control plants.

Table. III. Protein-, chlorophyll-, sugar-, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco)and content of coupling factor of photophosphorylation (CF_1) as well as fresh weight of leaves of the Chinese Tung-Oil Tree *Aleurites montana* under the influence of 14 days of 0.3 ppm SO_2 in air.

Aleurites montana	Fresh weight/area [mg/cm^2]	Protein/ area [mg/cm^2]	Rubisco/ area [mg/cm^2]	Chloro- phyll/area [mg/cm^2]	Chloro- phyll *a/b*	Sugar/area [mg/cm^2]	Sugar % of protein	Rubisco % of protein	CF_1 % of protein	Chloro- phyll % of protein
Control plants	18.7	1.74	1.01	0.10	3.33	0.40	22.8	58.7	4.0	5.72
SO_2-plants	17.4	1.28	0.65	0.037	2.93	0.14	11.0	50.6	5.7	2.83

Control plants were 9 months old and then exposed for 14 days to 0.3 ppm SO_2 in air. Chlorophyll and protein determinations deviated by 2 to 3%.

As the protein content referred to leaf area also suffers a reduction during this 14 days lasting experiment, it appears that the reference finally should rather be the leaf area since the leaf area stays constant. Referred to leaf area, the chlorophyll content, Rubisco and soluble sugars suffer a drastic reduction which clearly demonstrates the detrimental effect of SO_2 on *Aleurites* plants.

The chlorophyll *a/b* ratio shifts from 3.33 to 2.93. As chlorophyll *b* occurs only in the light antenna, an increase of chlorophyll *b* might mean an increase of the light harvesting complex relative to the other peptides. This is confirmed by the analyses of isolated PS II particles by SDS-PAGE. As shown in Fig. 4 and confirmed by densitometric analysis of the electrophoretic bands, the bands of the light harvesting complex peptides are stronger in SO_2-plants. In parallel, the chlorophyll-binding peptides with the molecular masses 43 and 47 kDa increase, whereas the extrinsic PS II-peptides with the molecular masses of 33, 21 and 16 kDa occur in lower concentration. This increase of the LHCP-complex in SO_2-plants was also confirmed by Western blot.

Simultaneous effect of SO_2 and high CO_2

In a third experiment we have exposed 10 months old plants of *Aleurites* to an atmosphere which contained 0.3 ppm SO_2 and 700 ppm CO_2. A phenotypical comparison showed that in this case only approx. 10% of the older leaves of these plants developed brown spots/areas, which then led to a shedding of these leaves. The majority of the leaves, however, showed no visual differences in comparison to those of control plants. Also the newly expanding leaves seemed to adapt to the new condition of an increased SO_2- and CO_2-content during the 14 days lasting experimental period.

In agreement with the visual appearence of the plants, the comparative analyses of the SO_2-/CO_2-plants and the corresponding control plants show that the *detrimental* influence of SO_2 is releaved by the simultaneous gassing with 700 ppm CO_2. With reference to leaf area the protein content appears increased by approx. 27% and the chlorophyll content appears increased by 33%. Even the content of soluble sugars and that of Rubisco increase by 14 and 18% respectively (Table IV). Referred to soluble proteins, however, the chlorophyll, Rubisco and sugar content decreases. Even the CF_1-content decreases in these plants and behaves like in CO_2-plants (Table IV). Only with SO_2-plants a 43% increase of the CF_1-content referred to proteins was observed (Table III).

As the chlorophyll content increases by 33% it could be concluded that either the number of chlo-

Table IV. Protein-, chlorophyll-, sugar-, ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) and content of coupling factor of photophosphorylation (CF_1) as well as fresh weight of leaves of the Chinese Tung-Oil Tree *Aleurites montana* cultivated for 14 days in air containing 0.3 ppm SO_2- and 700 ppm CO_2 and comparison to the values of plants grown under normal air conditions.

Aleurites montana	Fresh weight/area [mg/cm^2]	Protein/ area [mg/cm^2]	Rubisco/ area [mg/cm^2]	Chloro- phyll/area [mg/cm^2]	Chloro- phyll *a/b*	Sugar/area [mg/cm^2]	Sugar % of protein	Rubisco % of protein	CF_1 % of protein	Chloro- phyll % of protein
Control plants	15.0	2.31	0.71	0.043	3.22	0.49	24.3	34.5	5.1	2.11
SO_2/CO_2-plants	16.0	2.94	0.84	0.057	3.28	0.56	22.0	28.6	4.6	1.97

Control plants were 10 months old and then exposed to the combined mixture of 700 ppm CO_2 and 0.3 ppm SO_2 in air. Chlorophyll and protein values deviated 2 to 3%.

roplasts or the number/amount of LHCP-complexes in the chloroplasts has increased. SDS-gel electrophoretic analyses show that the LHCP-complex has increased. As the chlorophyll *a/b* ratio remains practically constant it is concluded that the increase of the LHCP-complex refers to PS II but has been compensated by a decrease of the LHCP-complex in photosystem I. The proof for this interpretation must come from future work. In the PS II-complex also an increase of the chlorophyll-binding peptides with the molecular masses 43 and 47 kDa as well as that of the extrinsic peptide with the molecular mass of 33 kDa was observed. The peptides with molecular masses smaller than 24 kDa decreased as well as the dimer of the D1 peptide with the molecular mass of 66 kDa.

Discussion

The present studies on the influence of gaseous compounds as CO_2 and SO_2 on growth of *Aleurites* were conducted in such a way as to cover the entire vegetation period after three months of normal growth after germination. *Aleurites fordii* and *montana* are multiannual arboreous plants. Under the influence of increased CO_2 (700 ppm) in the atmosphere, the plants clearly exhibit an increased growth (Fig. 1). After 4 months exposure to the increased CO_2-content the plants have built up 30–40% more biomass than control plants (Fig. 1). Such results are known for higher plants for some time. It is, however, important to emphasize that the increased growth was sustained over the entire 4 months period. However, the influence of SO_2, although only applied in the low concentration of 0.3 ppm in air over 14 days, appeared detrimental to the plants. In this case, it should be noted that the detrimental influence referred to older fully expanded leaves which after 6 days of SO_2-treatment exhibited brown leaf areas, so-called leaf necrosis (Elstner and Hippeli, 1995). Young leaves expanding under the influence of SO_2 did not exhibit this decomposition at least not with 0.3 ppm SO_2. Cultivation of plants under the simultaneous exposure of 0.3 ppm SO_2 and increased CO_2 (700 ppm), at least, as jugded from the appearence of the plants and the photosystem II peptide analyses, diminished the noxious influence of SO_2 observed in the presence of a normal CO_2-content in air.

As with higher plants leaves in dependence of the leaf position are exposed to different light intensities, we distinguished between sun and shade leaves.

The bifunctional enzyme ribulose-1.5-phosphate carboxylase/oxygenase and the coupling factor of photophosphorylation were analyzed by means of monospecific polyclonal antisera via a quantitative immune electrophoresis procedure namely that of rocket immune electrophoresis (Radunz and Schmid, 1988; Nespoulous *et al.*, 1988; Radunz and Schmid, 1989; Schmid *et al.*, 1993). This precise and very specific method permits a direct quantitative determination of these components in the presence of other proteins and enzymes, without any previous protein precipitation or concentration, which usually leads to losses and/or denaturation.

In young plants of *Aleurites montana*, Rubisco makes up for approx. 30% of the soluble proteins but does not stay constant during the vegetation period. It increases with the age of the plants to 50–60% of the leaf proteins. In CO_2-plants Rubisco is at first present in higher concentrations than in the corresponding control plants. However, in CO_2-plants the enzyme decreases stronger after a 3 months cultivation under these conditions. The decrease might comprise up to 50% of the Rubisco amount of control plants. As *Aleurites* plants grown under increased CO_2 constantly increase their biomass in the same time by 40% it is concluded that the activity of the Rubisco is increased. Using antisense rbcs transformed *N. tabacum* plants Stitt and Schulze (1994) also conclude that in these transformants which contain lower levels of Rubisco, the enzyme works more efficiently. However, it remains unclear whether experiments with gene-manipulated plants are comparable with the observed adaptation of higher plants to increased CO_2-conditions.

After four weeks cultivation under increased CO_2 (700 ppm) *Nicotiana tabacum* plants, analyzed in parallel to these experiments, contain Rubisco in a lower concentration. With the *N. tabacum* Su/su mutant, however, in which Rubisco has generally a higher oxygenase activity (Ishii and Schmid, 1982) Rubisco is present in higher concentrations during the first three weeks under these conditions. The comparison between other plants shows that the Rubisco content in depen-

Table V. Comparison of the molar ratio of coupling factor of photophosphorylation (CF_1) and rubisco to chlorophyll and ratio of coupling factor to Rubisco in *Aleurites* plants, which had been cultivated at different CO_2- and SO_2-concentration in air. The values are taken from Tables I, III and IV.

Aleurites montana	Age of plants/ duration of CO_2-/SO_2- gassing in months	CF_1/Chlorophyll	Rubisco/Chlorophyll	CF_1/Rubisco
Control plants	5/0	1/303	1/99	1/3.1
CO_2-plants	5/2	1/395	1/80	1/4.9
Control plants	6/0	1/191	1/52	1/3.7
CO_2-plants	6/3	1/396	1/89	1/4.5
Control plants	7/0	1/322	1/32	1/10.2
CO_2-plants	7/4	1/326	1/54	1/6.0
Control plants	9/0	1/395	1/36	1/11.0
SO_2-plants	9/14 days	1/269	1/35	1/7.7
Control plants	10/0	1/154	1/34	1/4.6
SO_2-/CO_2-plants	10/14 days	1/201	1/42	1/4.8

dence on the CO_2-content might vary differently. In *Lycopersicum esculentum*, another member of Solanaceae, Rubisco was reduced to almost half as consequence of growth under increased CO_2 (Besford *et al.*, 1990). With *Pinus taeda* under these conditions the amount as well as the activity decreases (Tissue *et al.*, 1993). Campbell *et al.* (1988) describe for soybeans that concentration and activity of Rubisco stay unchanged. Sage *et al.* (1989) found for 5 different C_3-species, namely for *Chenopodium album, Brassica oleracea, Solanum tuberosum, Solanum melongena* and *Phaseolus vulgaris* that the activity of Rubisco under concentrations of increased CO_2 is decreased. Whereas in the first two species also the concentration of the enzyme is decreased, the Rubisco concentration stays constant with the other species.

The coupling factor of photophosphorylation which is responsible for the formation of ATP, stays during development and the entire vegetation period of *Aleurites* plants practically constant. However, under the influence of increased CO_2 the amount of CF_1 decreases. In experiments with *Nicotiana tabacum* carried out in parallel to the *Aleurites* experiments it is seen that the CF_1 content in tobacco is twice as high as in *Aleurites* plants. In tobacco CF_1 makes up for 10% of total soluble proteins (Radunz and Schmid, unpublished). After a 3–4 weeks cultivation under increased CO_2 (700 ppm) CF_1 is also reduced in *Nicotiana tabacum* plants. Whether the reduction of CF_1 in CO_2-plants is only due to a morphological alteration of the chloroplast structure (exten-

sion of grana regions) (Radunz and Schmid, 1988) must be verified by future work.

In Table V the molar ratio of CF_1 to Rubisco is compared in *Aleurites* plants. It is seen that in young plants the ratio is 1:3 and in older plants 1:10. It is also seen that the ratio of CF_1 to Rubisco is bigger in a CO_2-plant. Hence, the ratio is shifted in favor of Rubisco.

SDS-polyacrylamide gel electrophoresis for the analysis of peptides led to the result that an increase of the CO_2-content in air to 700 ppm leads in chloroplasts as well as in PS II-particles to quantitative alterations of the peptide compostion (Fig. 4). The LHCP-complex (measured as peptide) increases after a 4 months cultivation of the plants under increased CO_2. Also the peptide of the oxygen evolving complex with the molar mass of 33 kDa occurs in higher concentration. At first glance, a discrepency to this data seems to exist with respect to the chlorophyll values and the chlorophyll *a/b* ratio of leaves and those of isolated chloroplasts and PS II particles (Table II). From the observation that the chlorophyll content of CO_2-plants is always lower than that of control plants and from the observation that the chlorophyll *a/b* ratio is always higher in CO_2 plants it can be concluded (as chlorophyll *b* occurs only in the LHCP) that the LHCP, taken as quantity of chlorophyll, is reduced/ decomposed in CO_2-plants. However, as in isolated chloroplasts and in PS II-particles the chlorophyll *a/b* ratio is smaller in CO_2-plants in comparison to control plants (Table II) and as, furthermore, the CF_1 to chlorophyll ra-

tio is higher in CO_2-plants (Table V), this means that the number of LHCP units with a reduced chlorophyll content/unit is increased in CO_2-plants. The densitometric analysis of the gels confirms the result of the PS II-peptide analysis.

In *Nicotiana tabacum* plants which have been grown for 3 weeks under an increased CO_2-concentration (700 ppm) we observed an increase of LHCP-peptides, here, however, connected with a general increase of the chlorophyll content (Radunz and Schmid, unpublished). In PS I of these *Nicotiana tabacum* plants, however, the increased CO_2-content of air leads to a decrease of LHCP and an increase of CPI-core peptides (Makewicz *et al.*, 1995b,c). It looks as if in most cases plants react to the high CO_2-conentration in air in the same or in a similar way as when the light intensity is high over a prolonged time period. In this case the photosynthetic unit is reduced (Schmid and Gaffron, 1967; Homann and Schmid, 1967; Wild *et al.*, 1973).

After a two weeks treatment of the plants with 0.3 ppm SO_2 in air, the molecular structure of photosystem II is drastically changed. Although the light-harvesting complex increases in comparison to control plants, this increase is contrasted by a reduction of the peptides of the oxygen evolving complex, with the molar masses of 33, 21 and 16 kDa as well as a reduction of the chlorophyll-binding peptides (of the proximal antenna of PS II) with the molecular masses of 47 and 43 kDa and of all peptides with molecular masses lower than 18 kDa. Although the plant increases its light harvesting capacity, it cannot use it, as the oxygen-evolving complex has suffered serious damage leading to a reduction of electron transport reactions with the consequence that the production of $NADPH^+$ and ATP is reduced. Here, storage and reserve components such as soluble proteins and soluble sugars occur in only reduced amounts.

The simultaneous gassing/treatment of the plants with SO_2 (0.3 ppm) and CO_2 (700 ppm) leads to a certain extent to a compensation of the harmful influence of SO_2. Thus, the LHCP-peptides are increased and peptides of the oxygen-evolving complex correspond to those of control plants at least as far as the 33 kDa and 21 kDa peptides are concerned. Parallel to this situation the content of soluble sugars and proteins, referred to leaf area, is increased. The chlorophyll content somewhat increased in comparison to the control plants due to the higher LHCP-content.

The leaf lipids of CO_2-plants of *Aleurites* contain 14% more chlorophyll, 8% more phospholipids and 6.5% more carotenoids. Galactolipids are reduced by approx. 8%. The fatty acid composition is characterized by a higher portion of saturated fatty acids. In the seeds the unusual octadecatriene-acid is present, having conjugated double bounds and a Δ^9 *cis*-, Δ^{11} *trans*- and Δ^{13} *trans*-configuration which is missing in the listed leaf lipids. Details of the lipid- and fatty acid-changes under the increased CO_2-content will be reported in a separate publication (He *et al.*, in preparation).

Acknowledgement

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