



*Anna Szadel, Gabriela Lorenc-Plucińska,  
Piotr Karolewski, Renata Matysiak*

## Photochemical activity, photosynthetic pigments and carbohydrates in poplar leaves fumigated with sulphur dioxide

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**Abstract:** The purpose of this study was to assess the influence of SO<sub>2</sub> on photosynthetic apparatus and the level of total nonstructural carbohydrates (TNC) in developing and mature leaves of poplar (*Populus deltoides*). Photosynthetic apparatus was evaluated on the basis of fluorescence parameters ( $F_v/F_m$ ,  $\Phi_{PSII}$ ,  $q_p$  and  $R_{fd}$ ) and photosynthetic pigments (chlorophylls and carotenoids). Cuttings of poplar were exposed to 0.25 ppm of SO<sub>2</sub> at 25°C and 200–300 mmol m<sup>-2</sup>s<sup>-1</sup> PAR for 6 hours daily during 5 days in a fumigation chamber. The fumigation did not produce any significant differences in fluorescence parameters in neither developing nor mature leaves. In some mature leaves the concentration of pigments increased under the influence of SO<sub>2</sub>. Soluble carbohydrates decreased significantly both in developing and mature leaves and this was accompanied with an increase in starch accumulation. We suggest that *Populus deltoides* is a species tolerant to sulphur dioxide and the used SO<sub>2</sub> dosage did not significantly impair the light reactions of photosynthesis, but it disturbed the accumulation of starch and the utilization of soluble carbohydrates in plants exposed to SO<sub>2</sub>.

**Additional key words:** carotenoids, chlorophylls, *Populus deltoides*, sugars

**Address:** A. Szadel, G. Lorenc-Plucińska\*, P. Karolewski, R. Matysiak,  
Polish Academy of Sciences, Institute of Dendrology, ul. Parkowa 5, 62-035 Kórnik, Poland.  
\*corresponding author: e-mail: glp@man.poznan.pl

**Abbreviations:**  $\Phi_{PSII}$  – efficiency of PSII photochemistry,  $F_v/F_m$  – maximum quantum efficiency of PSII,  $q_p$  – photochemical quenching coefficient, LPI – leaf plastochron index,  $R_{fd}$  – vitality index, TNC – total nonstructural carbohydrates, X+car – carotenoids (xanthophylls + carotens)

### Introduction

Harmful effects of SO<sub>2</sub> on plants have been described as changes in the level of photosynthetic pigments, altered rate of photosynthesis, and loading of sucrose into phloem (Wellburn 1985; Maurousset et al. 1992; Lorenc-Plucińska et al. 2001).

Measurements of chlorophyll *a* fluorescence, concentration of leaf carbohydrates and their translo-

cation have been used in research on the impact of air pollution on plants (Lichtenthaler and Rinderle 1988; Bücker and Ballach 1992). The main interest of this study was to check the influence of SO<sub>2</sub> on photosynthesis, measured with fluorescence parameters, photosynthetic pigments and the level of total nonstructural carbohydrates (TNC).

The ratio of variable to maximal fluorescence ( $F_v/F_m$ ), as a sensitive indicator of photodamage, is

indicative of the maximum quantum efficiency of PSII photochemistry (Lorenzini et al. 1999). It is used as a screening parameter of stress response (Björkman and Demmig 1987). The efficiency of PSII photochemistry ( $\Phi_{PSII}$ ) measures the proportion of the light absorbed by chlorophyll associated with PSII that is used in photochemistry. The light energy partitioning within the photosynthetic apparatus can be detected by measuring the fluorescence photochemical quenching coefficient ( $q_p$ ). It gives an indication of the proportion of PSII centres that are open (Maxwell and Johnson 2000). The fluorescence ratio  $R_{fd}$ , termed vitality index, is used to present the overall photosynthetic function of the leaf, including the activity of the Calvin cycle and related processes (Mohammed et al. 1995).

## Materials and methods

**Plant material.** Rooted cuttings of *Populus deltoides* Bartr. ex Marsh were grown in a greenhouse in natural day/night cycles at 22/16°C and relative humidity of 60–80%. The seeds originated from poplar trees species growing on experimental plots at the Institute of Dendrology, Kórnik, Poland (52°15'N, 17°06'E). The plants were used in the experiments when the 16<sup>th</sup> leaf from the stem base reached 2 cm in length; the leaf was regarded as LPI 1. On such plants, leaves were divided into developing (LPI 1–6) and mature (LPI 7–16) on the basis of their position on the shoot (Ceulemans and Isebrands 1996).

**Experimental treatments.** The plants were exposed to 0.25 ppm of SO<sub>2</sub> in a fumigation chamber, at 25°C and 200–300 mmol m<sup>-2</sup>s<sup>-1</sup> PAR for 6 hours daily during 5 days. For dosing gas, the Interscan LD Continuous Monitoring System Model LD-24 Sulphur Dioxide (Canada) was used. Plants growing in chambers without SO<sub>2</sub> fumigation served as controls. Both types of cuttings (control and SO<sub>2</sub>-fumigated) were represented by five plants each. After the fumigation, chlorophyll *a* fluorescence was measured with a field-portable fluorometer (FMS 2, Hansatech UK) after 15 min in darkness. The vitality index ( $R_{fd}$ ) was calculated as  $R_{fd} = (F_m - F_s) / F_s$ , where  $F_m$  is maximum and  $F_s$  is steady-state fluorescence. The method described by Lichtenthaler and Wellburn (1983) was used for chlorophyll content determination. Soluble carbohydrates and starch levels were prepared as described by Hassig and Dickson (1979) and Hansen and Møller (1975), modified by Oleksyn et al. (1997). These measurements were repeated three times per one LPI. Statistical data analyses, including an analysis of variance, were conducted with JMP software (version 4.0.4, SAS Institute, Cary, NC).

## Results and discussion

No visible symptoms were observed after SO<sub>2</sub> fumigation of the leaves. The concentration of chlorophylls and carotenoids was higher in mature leaves than in developing leaves in control and fumigated plants (Fig. 1). There was also a significant interac-

Table 1. Analysis of variance of the effect of SO<sub>2</sub> treatment (T), age (A) and A × T interactions on fluorescence parameters and levels of chlorophylls, carotenoids, soluble carbohydrates and starch in leaves of *Populus deltoides*

	Chl(a+b)	X+car	F <sub>v</sub> /F <sub>m</sub>	PSII	q <sub>p</sub>	R <sub>fd</sub>	Soluble carbohydrates	Starch
Age (A)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment (T)	0.8695	0.0044	0.2320	0.1716	0.1629	0.5081	<0.0001	<0.0001
A × T	<0.0001	0.0002	0.6561	0.5829	0.5416	0.2732	<0.0001	<0.0001

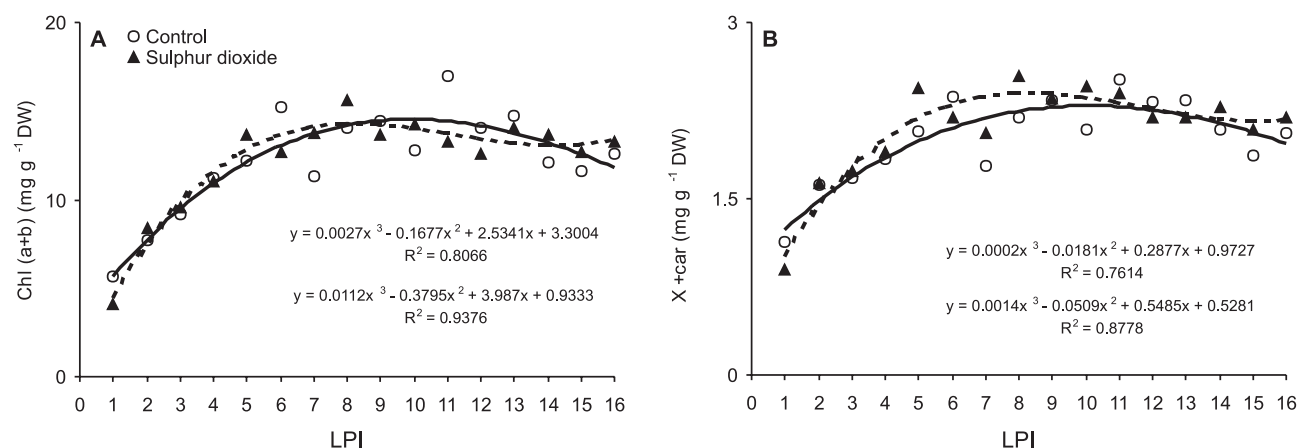


Fig. 1. Concentration of chlorophyll (a+b) (A) and carotenoids (xanthophylls + carotens, X+car) (B) in leaves (LPI 1–16) of *Populus deltoides* fumigated with SO<sub>2</sub> (dashed line) and of control plants (continuous line)

tion between SO<sub>2</sub> treatment and leaf age (Table 1). This confirms our previous finding (Lorenc-Plucińska et al. 2001) that photosynthetic activity is modified under the influence of SO<sub>2</sub> in relation to leaf age.

The values of the fluorescence parameters depended on leaf age (Table 1). The fumigation with SO<sub>2</sub> did not produce any significant differences in  $F_v/F_m$ ,  $\Phi_{PSII}$ ,  $q_P$  and  $R_{fd}$  in comparison to control plants,

both in developing and mature leaves (Table 1). There was no significant interaction between SO<sub>2</sub> treatment and leaf age (Table 1). These results are in contrast with the effect of O<sub>3</sub> on photochemical efficiency in developing and mature poplar leaves observed by Lorenzini et al. (1999), where there was an interaction between O<sub>3</sub> treatment and leaf age. This can be explained by the different place of action in the

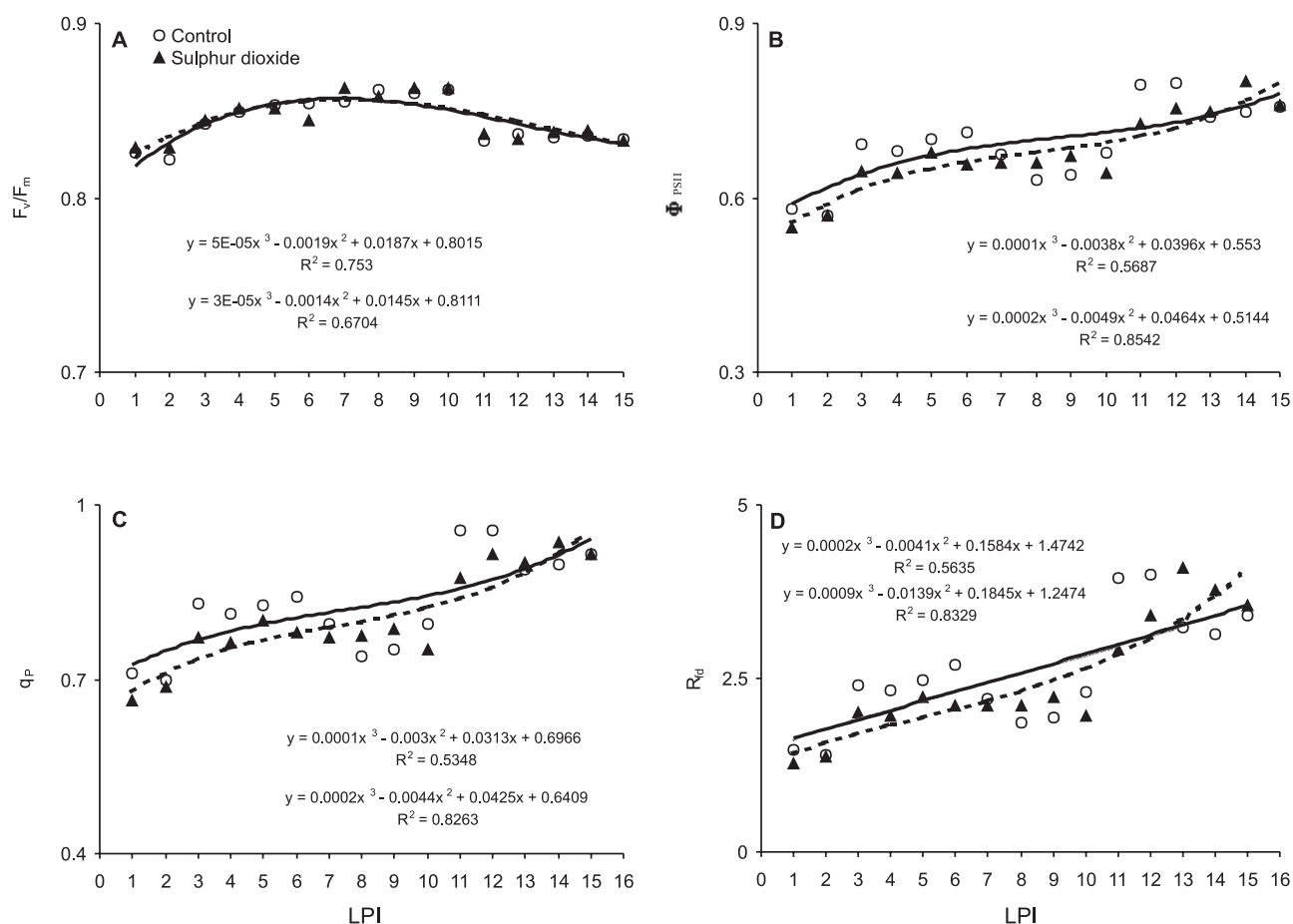


Fig. 2. Chlorophyll *a* fluorescence parameters:  $F_v/F_m$  (A),  $\Phi_{PSII}$  (B),  $q_P$  (C) and  $R_{fd}$  (D) in leaves (LPI 1–16) of *Populus deltoides* fumigated with SO<sub>2</sub> (dashed line) and of control plants (continuous line). The measurements were conducted at the end of fumigation, after 15 min dark adaptation of the leaves

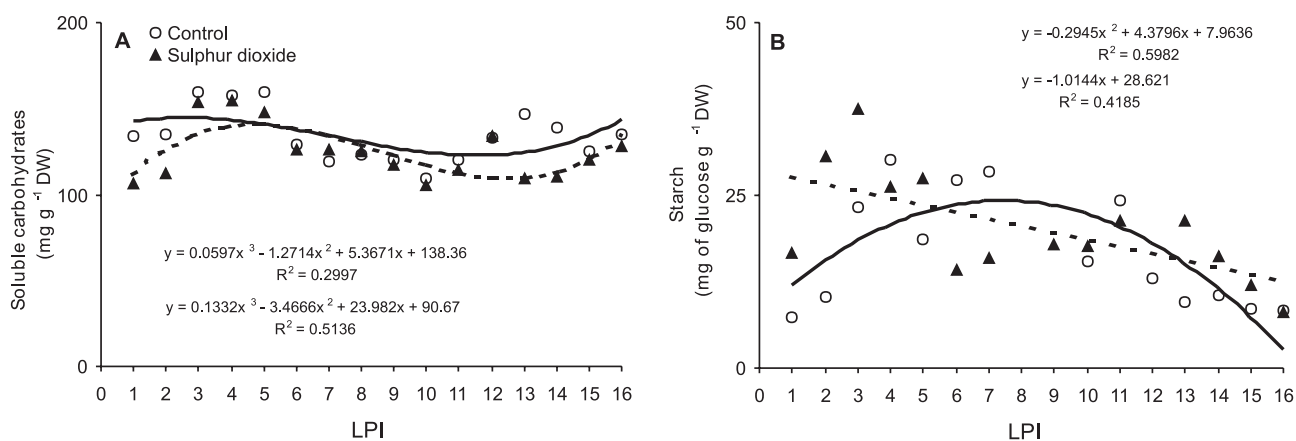


Fig. 3. Concentration of soluble carbohydrates (A) and starch (B) in leaves (LPI 1–16) of *Populus deltoides* fumigated with SO<sub>2</sub> (dashed line) and of control plants (continuous line)

cell, described by Alscher et al. (1997). An average value of 0.832 for  $F_v/F_m$  (Fig. 2A) is typical of well-functioning photosynthetic apparatus (Björkman and Demmig 1987). The value below 0.725 is regarded as photoinhibition, when plants are exposed to environmental stresses (Critchley 2000). Our results show that fumigation with  $SO_2$  does not alter the maximum photochemical yield of PSII centres (Fig. 2A) and  $\Phi_{PSII}$  (Fig. 2B). There was also no evidence for photoinhibitory damage of PSII centres (Fig. 2C). These data are consistent with other results of experiments on poplar leaves. In an  $O_3$ -resistant clone of poplar, ozone treatment had no significant effect on  $\Phi_{PSII}$  and  $q_p$  of developing leaves (Lorenzini et al. 1999) and  $F_v/F_m$  and  $q_p$  of mature leaves (Guidi et al. 1998). The vitality index ( $R_{fd}$ ) indicates the potential photosynthetic activity (Lichtenthaler and Rinderle 1988). The values of vitality index in poplar fumigated with  $SO_2$  (Fig. 2D) suggest that both developing and mature leaves showed a high potential photosynthetic activity (Lichtenthaler and Rinderle 1988; Mohammed et al. 1995). *Populus deltoides* is tolerant to sulphur dioxide and the used  $SO_2$  concentration did not significantly impair the light reactions of photosynthesis in treated plants. Chlorophyll fluorescence parameters (Fig. 2A-D) indicate no change in overall photosynthetic performance of *Populus deltoides*, as described earlier (Lorenc-Plucińska et al. 2002).

Under the influence of many stress factors, the primary effects of stress have been reported to occur mostly at the level of enzymatic dark reactions, and chlorophyll fluorescence is affected after other parameters (Mohammed et al. 1995). In poplar plants the level of soluble carbohydrates and starch was dependent on leaf age and  $SO_2$  treatment, and there was also a significant interaction between leaf age and  $SO_2$  treatment (Table 1). The level of soluble carbohydrates decreased due to fumigation with  $SO_2$  both in young and mature leaves (Fig. 3). The decrease in soluble sugars may be a consequence of increased metabolic consumption of energy under stress conditions (Bücker and Ballach 1992). The level of starch was increased in mature leaves treated with  $SO_2$  (Fig. 3). The enhanced starch accumulation in plants exposed to  $SO_2$  may result from a perturbation of sucrose loading into phloem or to the impaired transport of triose-phosphate across the plastid envelope (Lorenc-Plucińska 1998; Ranieri et al. 2000). The capacity of starch synthesis enables many plants to achieve a higher rate of photosynthesis when sucrose synthesis is restricted, because the synthesis of starch contributes to triose-phosphate utilization in the chloroplast (Paul and Foyer 2001).

On the basis of the results obtained in the present study, we suggest that the concentration of  $SO_2$  used

does not significantly impair the light reactions of photosynthesis. It disturbs the balance between synthesis of starch and soluble carbohydrates and the utilization of sugars in poplar leaves exposed to  $SO_2$ .

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