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

Received: 20 January 2003, Accepted: 11 April 2003

Abstract: The purpose of this study was to assess the influence of SO₂ 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 , Φ_{PSII} , q_p and R_{fd}) and photosynthetic pigments (chlorophylls and carotenoids). Cuttings of poplar were exposed to 0.25 ppm of SO₂ at 25°C and 200–300 mmol m⁻²s⁻¹ 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 SO₂. 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 SO₂ 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 SO₂.

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

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Abbreviations: Φ_{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 SO₂ 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 SO₂ 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 (Φ_{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 16th 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 SO₂ in a fumigation chamber, at 25°C and 200–300 mmol m⁻²s⁻¹ 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 SO₂ fumigation served as controls. Both types of cuttings (control and SO₂-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 SO₂ 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 SO₂ 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 _v /F _m	PSII	q _P	R _{fd}	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

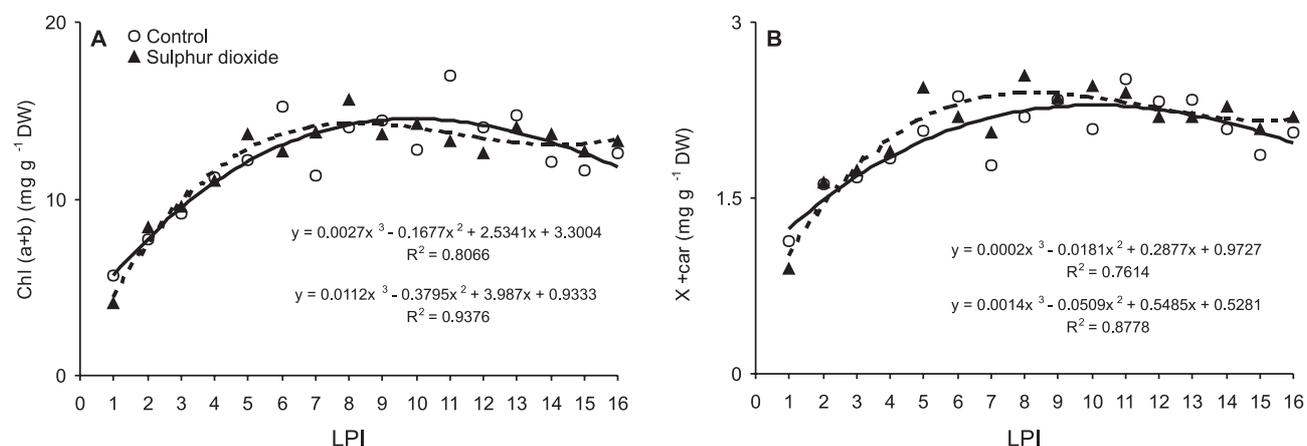


Fig. 1. Concentration of chlorophyll (a+b) (A) and carotenoids (xanthophylls + carotens, X+car) (B) in leaves (LPI 1–16) of *Populus deltoides* fumigated with SO₂ (dashed line) and of control plants (continuous line)

tion between SO₂ 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 SO₂ in relation to leaf age.

The values of the fluorescence parameters depended on leaf age (Table 1). The fumigation with SO₂ did not produce any significant differences in F_v/F_m , Φ_{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 SO₂ treatment and leaf age (Table 1). These results are in contrast with the effect of O₃ on photochemical efficiency in developing and mature poplar leaves observed by Lorenzini et al. (1999), where there was an interaction between O₃ treatment and leaf age. This can be explained by the different place of action in the

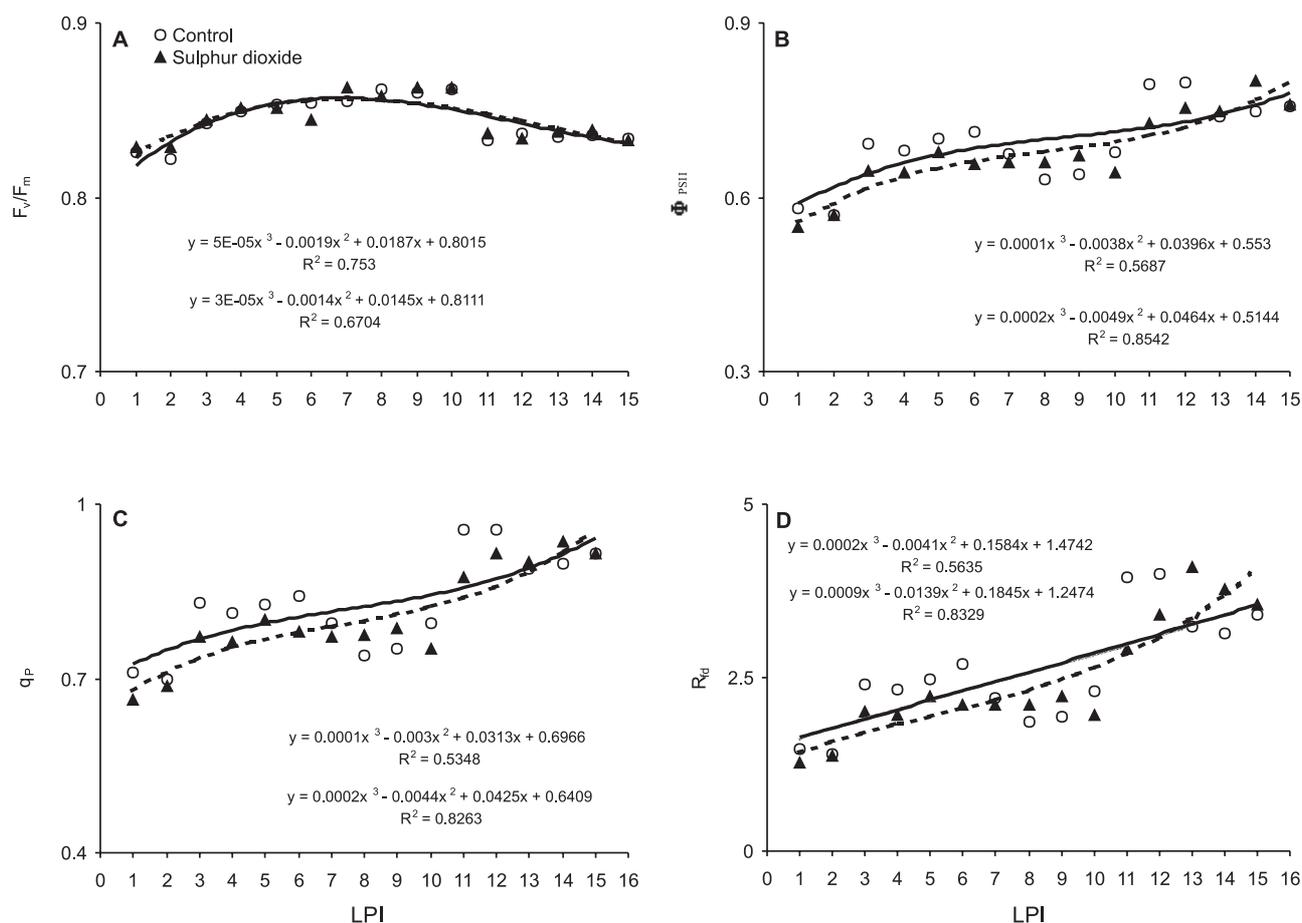


Fig. 2. Chlorophyll *a* fluorescence parameters: F_v/F_m (A), Φ_{PSII} (B), q_P (C) and R_{fd} (D) in leaves (LPI 1–16) of *Populus deltoides* fumigated with SO₂ (dashed line) and of control plants (continuous line). The measurements were conducted at the end of fumigation, after 15 min dark adaptation of the leaves

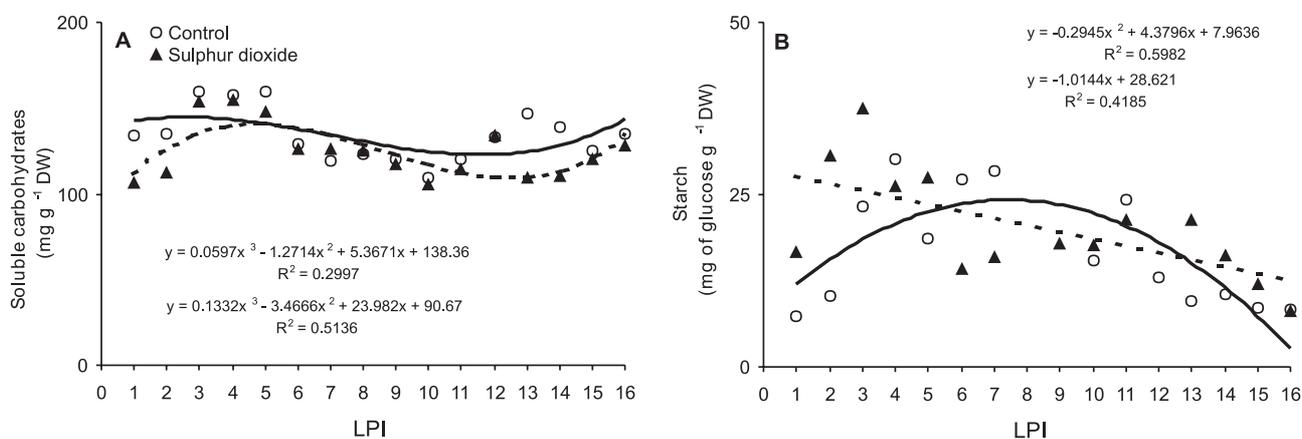


Fig. 3. Concentration of soluble carbohydrates (A) and starch (B) in leaves (LPI 1–16) of *Populus deltoides* fumigated with SO₂ (dashed line) and of control plants (continuous line)

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 Φ_{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 Φ_{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 .

Acknowledgements

This work was partly supported by grant No. 6 PO4G 057 16 and No. 6PO4 103 21 from the Polish State Committee for Scientific Research. We would like to acknowledge also K. Grewling, who provided excellent technical assistance.

References

- Alscher R.G., Donahue J.L., Cramer C.L. 1997. Reactive oxygen species and antioxidants: Relationships in green cells. *Physiologia Plantarum* 100: 224–233.
- Björkman O., Demmig B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 °K among vascular plants of diverse origins. *Planta* 170: 489–504.
- Bücker J., Ballach H.J. 1992. Alterations in carbohydrate levels in leaves of *Populus* due to ambient air pollution. *Physiologia Plantarum* 86: 512–517.
- Ceulemans R., Isebrands J.G. 1996. Carbon acquisition and allocation. In: *Biology of Populus and its Implications for Management and Conservation*. Stettler R.F., Bradshaw H.D., Heilman P.E., Hinckley T.M. (eds.). NRC Research Press, Ottawa, pp. 355–399.
- Critchley C. 2000. Photoinhibition. In: *Photosynthesis. A Comprehensive Treatise*. Raghavendra A.S. (eds.). Cambridge University Press, pp. 264–272.
- Guidi L., Nali C., Lorenzini G., Soldatini G.F. 1998. Photosynthetic response to ozone of two poplar clones showing different sensitivity. *Chemosphere* 36: 657–662.
- Hansen J., Møller I. 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. *Analytical Biochemistry* 68: 87–94.
- Hassig B.E., Dickson R.E. 1979. Starch measurement in plant tissue using enzymatic hydrolysis. *Physiologia Plantarum* 47: 151–157.
- Lichtenthaler H.K., Rinderle U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. *Critical Reviews in Analytical Chemistry* 19: 29–85.
- Lichtenthaler H.K., Wellburn R.R. 1983. Determination of total carotenoids and chlorophylls *a* and *b* of extracts in different solvents. *Biochemical Society Transactions* 603: 591–592.
- Lorenc-Plucińska G. 1998. The effect of sulphite on the regulation of photosynthetic sucrose synthe-

- sis in poplar leaves. *Journal of Experimental Botany* 49: 213–219.
- Lorenc-Plucińska G., Szadel A., Pluciński A., Pukacka S. 2001. Sucrose degradation in sink and source poplar leaves treated with sulfite. *Acta Societatis Botanicorum Poloniae* 3: 209–214.
- Lorenc-Plucińska G., Szadel A., Pluciński A., Matysiak R. 2002. The effect of sulphite on chlorophyll fluorescence and sucrose metabolism in poplar leaves. *Acta Physiologiae Plantarum* 24: 123–129.
- Lorenzini G., Guidi L., Nali C., Soldatini G.F. 1999. Quenching analysis in poplar clones exposed to ozone. *Tree Physiology* 19: 607–612.
- Maurousset L., Raymond P., Gaudillere M., Bonnemain J.L. 1992. Mechanism of the inhibition of phloem loading by sodium sulfite: effect of the pollutant on respiration, photosynthesis and energy charge in the leaf tissues. *Physiologia Plantarum* 84: 101–105.
- Maxwell K., Johnson G.N. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* 345: 659–668.
- Mohammed G.H., Binder W.D., Gillies S.L. 1995. Chlorophyll fluorescence: a review of its practical forestry applications and instrumentation. *Scandinavian Journal of Forest Research* 10: 383–410.
- Oleksyn J., Tjoelker M.G., Lorenc-Plucińska G., Konwińska A., Żytkowiak R., Karolewski P., Reich P.B. 1997. Needle CO₂ exchange, structure and defense traits in relation to needle age in *Pinus heldreichii* Christ – a relict of Tertiary flora. *Trees* 12: 82–89.
- Paul M.J., Foyer Ch.H. 2001. Sink regulation of photosynthesis. *Journal of Experimental Botany* 360: 1383–1400.
- Ranieri A., Serini R., Castagna A., Nali C., Baldan B., Lorenzini G., Soldatini G.F. 2000. Differential sensitivity to ozone in two poplar clones. Analysis of thylakoid pigment – protein complexes. *Physiologia Plantarum* 110: 181–188.
- Wellburn A.R. 1985. SO₂ effects on stromal and thylakoid function. In: Sulphur dioxide and vegetation. Winner W.E., Mooney H.A., Goldstein R.A. (eds.). California, Stanford University Press, pp. 133–147.

