SUCROSE DEGRADATION IN SINK AND SOURCE POPLAR LEAVES TREATED WITH SULFITE

GABRIELA LORENC-PLUCIŃSKA, ANNA SZADEL, ANDRZEJ PLUCIŃSKI, STANISŁAWA PUKACKA

Polish Academy of Sciences, Institute of Dendrology
Parkowa 5, PL-62-035 Kórnik, Poland

(Received: June 7, 2001. Accepted: July 17, 2001)

ABSTRACT

Sulfite at concentrations from 0.5 to 5.0 mM was supplied to illuminated, detached poplar (*Populus deltoides* Bartr. ex Marsh) leaves via the transpiration stream. Concentrations of chlorophyll *a* and *b*, carotenoids, glucose, fructose, sucrose and starch, and extractable specific activities of sucrose synthase (SuSy), acid invertase (AI) and neutral invertase (NI) were measured. In sink and source leaves chlorophylls and carotenoids appeared to be increased by sulfite at concentrations ranging from 0.5 to 5.0 mM. In source leaves the application of 5.0 mM and ≥ 2.5 mM sulfite led to a decrease in total nonstructural soluble sugars (sTNC) and starch, respectively. The specific activity of SuSy was enhanced by ≥ 0.5 mM sulfite. There was only a slight decline in the activity of AI and NI. In sink leaves sTNC decreased and starch increased under the influence of sulfite at concentrations ≤ 1.0 mM and ≥ 0.5 mM, respectively. The effect of sulfite on the activity of AI, NI and SuSy was negligible. However, in both development groups of leaves alterations in sucrose, glucose and fructose contents were observed. On the basis of the obtained results we postulate that sulfite changes the sucrolytic activities primarily in source leaves and only marginally in sink leaves.

KEY WORDS: *Populus deltoides* Bartr. ex Marsh, sink and source leaves, sucrose degradation, sulfite stress.

INTRODUCTION

The impact of SO₂ on plants ranges from invisible symptoms to visible symptoms and changes in the development, growth and productivity. After the entering of SO₂ into the leaf and dissolving in aqueous phases of leaf interior, protons and the highly reactive bisulfite and sulfite anions, are forming in the cells (Hüve et al., 2000).

Sulfite can alter the rate of photosynthesis, with associated changes in the level of the photosynthetic pigments (Wellburn, 1985) and in the rate of sucrose synthesis and loading into the phloem (Maurousset et al. 1992; Lorenc-Plucińska 1998). Little information is available on the effect of sulfite anions on sucrose metabolism and carbon allocation. Therefore our investigations were conducted to evaluate the influence of various sulfite concentrations on the activity of sucrose metabolizing enzymes and on the level of nonstructural carbohydrates in sink and source poplar leaves.

MATERIALS AND METHODS

Plant material

Populus deltoides Bartr. ex Marsh plants were grown as described previously (Lorenc-Plucińska 1998, 2000). In

short, hardwood cuttings were planted in 1.5-litre plastic pots in a mixture of garden soil and peat (1:1). All plants were grown in a greenhouse under natural day/night cycles at 22/14°C and relative humidity of 60-80%. They were used for the study when the 16th leaf from the stem base (leaf plastochron index, LPI) reached ca. 2 cm in length. Leaves 2 – 4 and 6 below the index leaf were selected for experiments.

Experimental treatments

Sulfite was supplied to the illuminated detached stem with two attached leaves, one developing leaf (LPI 2-4) and one mature leaf (LPI 6), via the transpiration stream. Stems with selected leaves were excised 5.5 h after the beginning of the light period. Immediately the stems were recut while submerged in degassed distilled water, then transferred into plastic vials containing 1 ml H₂O or 0.5, 1.0, 2.5 or 5.0 mM of Na₂SO₃ solution and illuminated (400 μ mol m⁻² s⁻¹ PAR) at 23-25°C for 90 min in moving air. The sulfite solution was prepared in distilled water. All feeding solutions were buffered at pH 7.0 with 1 mM HEPES-NaOH and exchanged for fresh solutions every 25 min. At the end of the treatment, leaf samples were transferred to liquid nitrogen in the light and stored in liquid N₂ or at -80°C until required. The amount of solution taken up into the stem with two leaves was estimated by the weight

lost from the cuvette plus stem as in Brauer et al. (1990). For each sulfite concentration single stems (with two leaves) from 3 different plants were placed in 3 separate vials. For further analysis developing or mature leaves were taken together and represented one replication of the experiment.

Enzyme extractions

All procedures were carried out at 0 to 4°C. Tissue preparation for enzyme extraction was performed according to the procedure of Copeland and de Lima (1992) with minor modifications. Two-gram frozen leaf samples (without the petiole and the mid-vein) were ground in a mortar with 5 ml grinding medium containing 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 2 mM DTT, 6% (w/v) PVP-40 and 1% (w/v) PEG-20M. The extract was filtered through Miracloth and centrifuged at 20 000 g for 20 min and the supernatant was desalted on BioRad Econo-Pac columns equilibrated with 50 mM Tris-HCl (pH 7.5).

Enzyme assays

Sucrose synthase (SuSy; EC 2.4.1.13) was assayed in the cleavage direction according to Huber (1989). Acid (AI) and alkaline (NI) invertases (EC 3.2.1.26) were assayed following the method reported by Sung et al. (1989). Proteins were estimated according to Bradford (1976), with bovine serum albumin as the standard.

Metabolite analysis

To extract soluble sugars (fructose, glucose, sucrose), leaf tissue was homogenized in a mixture of methanol, chloroform and water (12:5:3 v/v), and quantitatively determined using a HPLC system (Waters) fitted with a Sugar Pack I

column (Waters) and a refracting index detector (Waters). A detailed description of the procedure is given by Pukacka and Pukacki (1997). Total nonstructural carbohydrates (soluble and starch) were extracted and assayed as described by Oleksyn et al. (1997). The method reported by Lichtenthaler (1987) was used for chlorophyll determination.

Statistics

All experiments were repeated at least twice with a total of 6-9 replications. All data in the figures represent the mean values with standard errors not exceeding symbol size, except where indicated. Statistical data analyses included an analysis of variance with the *STATISTICA* software (StatSoft Inc., Tulsa, OK). Means were compared (between the control and sulfite treatment) by t-Tukey HSD (Tukey's honestly significant difference) test. Differences were considered significant at $P < 0.05$. For comparison between means of developing and mature leaves, the least significant difference (LSD) test was used.

RESULTS

Chlorophyll content

The differences in concentrations of chlorophylls and carotenoids (i.e. xanthophylls + carotens; X+car) between developing and mature leaves were significant, with developing leaves having lower concentrations than mature leaves (Fig. 1). Sulfite did not change this relationship (Fig. 1).

The application of sulfite at concentrations from 0.5 to 2.5 mM caused an increase in levels of the analysed pigments in mature leaves (Fig. 1). Developing leaves, in contrast, maintained their chlorophyll *b* content but chloro-

Fig. 1. Concentrations of chlorophyll *a*, chlorophyll *b*, chlorophylls *a+b* and carotenoids (xanthophylls + carotens, X+car) in leaves of *Populus deltoides*. Illuminated poplar stems with one developing and one mature leaf were supplied with water (control without sulfite) or sulfite (0.5-5.0 mM) via transpiration stream for 90 min. $\blacktriangle$ = sink leaves, $\bullet$ = source leaves. Each data point represents an average value ($\pm$ SE). Bars within the same series without a common letter (a, b, c) are significantly different from each other ($p < 0.05$). *, **, ***, **** = mean values for developing and mature leaves were significantly different at $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$, respectively.

Fig. 2. Concentrations of total nonstructural soluble sugars (sTNC), starch, sucrose, glucose, fructose, fructose/glucose ratio (fru/glu) and sucrolysis index (sucrose/hexose ratio) in leaves of *Populus deltoides*. Otherwise as in Fig. 1.

phyll *a* and carotenoid levels increased after applying 0.5 and 2.5 mM sulfite, respectively (Fig. 1).

Carbohydrate content

Concentrations of total nonstructural carbohydrates, soluble (sTNC) and insoluble (starch) were lower in developing leaves than in mature leaves (Fig. 2). This dependence occurred in control and sulfite-treated leaves (Fig. 2).

In mature leaves the concentrations of sTNC and starch decreased after applying 5.0 and ≥ 2.5 mM sulfite, respectively (Fig. 2). Feeding stems with sulfite at concentrations

≤ 1.0 mM led to a decrease in sTNC levels in developing leaves (Fig. 2). By contrast, the starch content increased under the influence of sulfite (Fig. 2).

Developing and mature leaves did not differ in sucrose content (Fig. 2) and sulfite application significantly lowered accumulation of this sugar in analysed leaves.

Developing and mature leaves had also similar levels of glucose (Fig. 2). After exposure to sulfite, however, the glucose content of mature leaves increased in comparison to developing leaves. The same dependence was observed in fructose content (Fig. 2). On the other hand, raffinose con-

Fig. 3. Specific activities of acid invertase (AI), neutral invertase (NI), sucrose synthase (cleavage direction, SuSy) and the total sucrolytic activity (TSA = AI + NI + SuSy) in leaves of *Populus deltoides*. Otherwise as in Fig. 1.

tent was higher in developing leaves and application of sulfite at concentrations ≤ 1.0 or 5.0 mM led to an increase or a decrease in raffinose accumulation, respectively (Fig. 2).

Enzyme activity

In developing and mature leaves the extractable specific activity of neutral invertase (NI) showed very low values, as compared with those of soluble acid invertase (AI) activity, while sucrose synthase (SuSy) activity assayed in sucrose cleavage direction was 7 times higher than the total invertase activity (AI plus NI) (Fig. 3). The specific activities of AI and SuSy as well as total sucrolytic activity (TSA) were significantly higher in developing than in mature leaves (Fig. 3).

In mature leaves specific activities of AI were only slightly affected by exposure to sulfite. In contrast, the activity of NI was increased by treatment with 5.0 mM sulfite (Fig. 3). SuSy also exhibited an increased activity after applying ≥ 1.0 mM sulfite (Fig. 3).

In developing leaves extractable activities of AI, NI, and SuSy and TSA remained nearly constant under the used sulfite concentrations (Fig. 3).

DISCUSSION

In poplar, sucrose is the major form of carbon transported from mature (source organs) to developing leaves (sink organs). Under the growing conditions of our study, poplar leaves at LPIs 2-4 are developing sink leaves importing assimilates produced in source leaves. The leaf at LPI 6 is considered a recently mature source leaf that transports photosynthates primarily to developing leaves (Vogelmann et al. 1982).

The sink-source transition is related to the alteration of the enzymatic activities and chlorophyll, protein, carbohydrate and amino acid concentrations. High levels of photosynthetic pigments (chlorophylls and carotenoids) and carbohydrates are typical for functional source leaves. Growing sink leaves are characterized by the activity of key enzymes, such as sucrose synthase and invertases, that play a regulatory function in phloem unloading and sucrose degradation and utilization (Roitsch 1999; Sturm and Tang 1999). The activities of enzymes involved in sucrose breakdown are also used as biochemical markers for sink strength (Herbers and Sonnewald 1998).

Organ-specific parameters for sink-source transition were noted in our study in leaves untreated with sulfite. Concentrations of chlorophylls, carotenoids and total non-structural carbohydrates were higher in mature source leaves, as compared with developing sink leaves (Figs 1 and 2). In contrast, extractable specific activities of acid invertase (AI), neutral invertase (NI) and sucrose synthase (SuSy) as well as the total sucrolytic activity (TSA) were higher in sink leaves (Fig. 3).

In leaves of many woody species AI was the primary enzyme responsible for sucrose hydrolysis (Schaffer et al. 1987; Einig et al. 1997). In poplar leaves the extractable specific activity of SuSy was about 8 (in sink leaves) and 6 (in source leaves) times higher than AI activity (Fig. 3), suggesting that SuSy was the major enzyme for sucrose breakdown. However, the fact that the ratio of fructose/glucose was about 0.6 in sink and 0.8 in source leaves (Fig. 3) seems to indicate that AI rather than SuSy contributed to reducing sugar pools. Fructose/glucose ratio is close to unity or is > 1.0 when sucrose degradation occurs via AI or SuSy, respectively (Egger et al. 1996). On the other hand, it cannot be excluded that the imbalance of fructose and

glucose resulted from starch breakdown (Bucker and Ballach, 1992; Egger et al. 1996).

Sulfite at concentrations < 5.0 mM or 5.0 mM increased or did not cause any noticeable changes in levels of the photosynthetic pigments, respectively (Fig. 1). This seems to indicate that photosynthetic capacity was increased or only slightly modified by sulfite. In spite of this, the changes in the pools of nonstructural carbohydrates and in the activity of enzymes responsible for sucrose hydrolysis suggest an alteration in sucrose metabolism, especially in source leaves. A lower susceptibility of young leaves of poplar in comparison with older ones was also observed in previous studies concerning the effect of SO₂, O₃ and NO₂ on carbon partitioning between different carbohydrates (Bucker and Ballach 1992; Bucker and Guderian 1993; Fialho and Bucker 1996).

The application of sulfite at concentrations ≥ 0.5 mM led to a decline in sucrose and starch contents of source leaves, with a corresponding significant increase in the specific activities of SuSy and TSA (Fig. 3) and a large reduction in the sucrolytic index (sucrose/hexoses ratio) (Fig. 3).

It is rather doubtful that a decreased level of starch in mature leaves after the application of sulfite (Fig. 2) was a consequence of lowered photosynthetic capacity, and the increases in chlorophylls (Fig. 1) confirm this conclusion. It could result from an increase in carbon allocation or a higher requirement for energy for matching maintenance and repair (Bücher and Ballach 1992; Fialco and Bücher 1996).

The activation of SuSy in source leaves by sulfite treatment may reflect a limited sucrose availability, an increase in supplying energy for loading and unloading in phloem or the demand for sugar translocation and the UDP-Glc yielding for biosynthesis route (Nolte and Koch 1993; Pelah et al. 1997). It is known that the action of sulfite inhibits the rate of sucrose synthesis and accumulation, and affects the loading of sucrose into the conducting complex and phloem unloading (Maurousset et al. 1992; Lorenc-Plucińska 1996, 1998, 2000). Sulfite can also lead to the inhibition of synthesis of ATP, followed by a lowering of the energy status of tissues (Wellburn 1985; Lorenc-Plucińska et al. 1989).

On the basis of the results obtained in the present study we postulate that sulfite changes also the sucrolytic activities, primarily in source leaves. In sink leaves sucrose metabolism was only marginally altered and may therefore support sink activities and growth capacity.

ACKNOWLEDGEMENTS

This work was partially supported by Grant No. 6 P04G 057 16 from the State Committee for Scientific Research, Poland. Thanks are also due to K. Grewling for her excellent technical assistance.

LITERATURE CITED

- BRADFORD M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Bioch.* 72: 248-254.
- BRAUER M., SANDERS D., STITT M. 1990. Regulation of photosynthetic sucrose synthesis: a role for calcium. *Planta* 182: 236-243.
- BUCKER J., BALLACH H.J. 1992. Alterations in carbohydrate levels in leaves of *Populus* due to ambient air pollution. *Physiol. Plant.* 86: 512-517.
- BUCKER J., GUDERIAN R. 1993. Marked increases in raffinose in leaves of *Populus* due to ambient air pollution. *J. Plant Physiol.* 141: 654-656.
- COPELAND L., DE LIMA M.L. 1992. The effect of aluminium on enzyme activities in wheat roots. *J. Plant Physiol.* 140: 641-645.
- EINIG W., LAUXMANN U., HAUCH B., HAMPP R., LANDOLT W., MAURER S., MATYSSEK R. 1997. Ozone-induced accumulation of carbohydrate changes enzyme activities of carbohydrate metabolism in birch leaves. *New Phytol.* 4: 673-680.
- EGGER B., EINIG W., SCHLERETH A., WALLEND T., MAGEL E., LOEWE A., HAMPP R. 1996. Carbohydrate metabolism in one- and two-year-old spruce needles, and stem carbohydrates from three months before until three months after bud break. *Physiol. Plant.* 96: 91-100.
- FIALHO R.C., BUCKER J. 1996. Changes in levels of foliar carbohydrates and myo-inositol before premature leaf senescence of *Populus nigra* induced by a mixture of O₃ and SO₂. *Can. J. Bot.* 74: 965-970.
- HERBERS K., SONNEWALD U. 1998. Molecular determinants of sink strength. *Curr. Opin. Plant Biol.* 1: 207-216.
- HUBER S.C. 1989. Biochemical mechanism for regulation of sucrose accumulation in leaves during photosynthesis. *Plant Physiol.* 91: 656-662.
- HÜVE K., VEJOVIC-JOVANOVIC S., WIESE C., HEBER U. 2000. Oxygen and light accelerate recovery from SO₂-induced inhibition of leaf photosynthesis and from cytoplasmic acidification. *J. Plant Physiol.* 156: 537-544.
- LICHTENTHALER H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* 148: 350-382.
- LORENC-PLUCIŃSKA G. 1989. Sugar transport and sulfite action in etioprotoplasts, semi-etiprotoplasts and green protoplasts of *Avena sativa* L. *Bot. Acta* 102: 37-45.
- LORENC-PLUCIŃSKA G. 1996. Sulfite inhibition of sucrose transport into vacuoles of cottonwood leaves. *Acta Soc. Bot. Pol.* 65: 291-295.
- LORENC-PLUCIŃSKA G. 1998. The effect of sulphite on the regulation of photosynthetic sucrose synthesis in poplar leaves. *J. Exp. Bot.* 49: 213-219.
- LORENC-PLUCIŃSKA G. 2000. The effect of SO₂ on sucrose efflux from source leaves of poplar. *Dendrobiology* 42: 61-64.
- 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. *Physiol. Plant.* 84: 101-105.
- NOLTE K., KOCH K.E. 1993. Companion-cell specific localization of sucrose synthase in zones of phloem loading and unloading. *Plant Physiol.* 101: 899-905.
- OLEKSYN J., TJOELKER M., 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.
- PELAH D., WANG W., ALTMAN A., SHOSEYOV O., BARTELS D. 1997. Differential accumulation of water stress-related proteins, sucrose synthase and soluble sugar in *Populus* species that differ in their water stress response. *Physiol. Plant.* 99: 153-159.
- PUKACKA S., PUKACKI P.M. 1997. Changes in soluble sugars in relation to desiccation tolerance and effects of dehydration on freezing characteristics of *Acer platanoides* and *Acer pseudoplatanus* seeds. *Acta Physiol. Plant.* 19: 147-154.
- ROITSCH T. 1999. Source-sink regulation by sugar and stress. *Curr. Opin. Plant Biol.* 2: 118-205.

- SCHAFER A.A., SAGEE O., GOLDSCHMIT E.E., GOREN R. 1987. Invertase and sucrose synthase activity, carbohydrate status and endogenous IAA levels during *Citrus* leaf development. *Physiol. Plant.* 69: 151-155.
- STURM A., TANG G.Q. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends Plant Sci.* 4: 401-407.
- SUNG S.S., KORMANIK P.P., XU D.P., BLACK C.C. 1989. Sucrose metabolic pathways in sweetgum and pecan seedlings. *Tree Physiol.* 5: 39-52.
- VOGELMANN T.C., LARSON P.R., DICKSON R.E. 1982. Translocation pathways in the petioles and stem between source and sink leaves of *Populus deltoides* Bartr. ex Marsh. *Planta* 156: 345-358.
- WELLBURN A.R. 1985. SO₂ effects on stromal and thylakoid function. In: Sulfur dioxide and vegetation. Winner W.E., Mooney H.A., Goldstein R.A. (ed.). California: Stanford University Press, pp. 133-147.

WPLYW JONÓW SIARCZYNOWYCH NA DEGRADACJĘ SACHAROZY W LIŚCIACH TOPOLI, DONORACH I AKCEPTORACH ASYMLATÓW

STRESZCZENIE

Odcięty pęd z dojrzałym (donor asymilatów) i rozwijającym się (akceptor asymilatów) liściem traktowano jonami siarczynowymi w stężeniu od 0.5 mM do 5.0 mM. Mierzono stężenie chlorofilu, karotenoidów, glukozy, fruktozy, sacharozy i skrobi oraz specyficzną aktywność syntazy sacharozy, kwaśnej i neutralnej inwertazy w liściach o określonym stopniu rozwoju. Pod wpływem działania jonów siarczynowych obserwowano wzrost stężeń chlorofilu i karotenoidów, niezależnie od stopnia rozwoju liści i pełnionej funkcji. W liściach – donorach asymilatów, jony siarczynowe powodowały obniżenie całkowitej zawartości cukrów niestrukturalnych, wzrost specyficznej aktywności syntazy sacharozy i statystycznie istotną deaktywację obu inwertaz. W liściach – akceptorach asymilatów, działanie jonów siarczynowych prowadziło do obniżenia całkowitej zawartości rozpuszczalnych cukrów niestrukturalnych i wzrostu skrobi oraz jedynie niewielkich zmian w aktywności analizowanych enzymów. W obu grupach liści (donor–akceptor) obserwowano także zmiany w stężeniach glukozy, fruktozy i sacharozy. Na podstawie otrzymanych wyników postuluje się, że jony siarczynowe wpływają przede wszystkim na aktywność sacharolityczną liści – donorów asymilatów.

SŁOWA KLUCZOWE: *Populus deltoides*, degradacja sacharozy, działanie siarczynu.