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SO_2 modification of sugar movement in source leaves of *Robinia pseudoacacia* L.*

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Abstract. The assimilation of $^{14}CO_2$, the mechanism of sucrose transport and the influence of various concentrations of sulfite on these activities was studied in source leaf tissues of black locust. Sulfite at concentrations $\geqslant 10.0$ mM inhibited $^{14}CO_2$ fixation. Uptake of sucrose was inhibited at concentrations of sulfite >2.5 mM. This latter inhibition was of the non-competitive type with a $K_i = 6.3$ mM of sulfite (25°C). CCCP reduced sucrose uptake in the control (without sulfite), completely eliminated the effect of sulfite at concentrations <10.0 mM, and significantly increased the inhibition of this process by 10.0 mM sulfite.

Additional key words: $^{14}CO_2$ assimilation, ^{14}C-sucrose uptake.

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INTRODUCTION

The distribution of CO_2 assimilation products throughout the plant in a systematic way is the basic condition for proper growth and development. The fulfillment of this condition is possible through continuous and coordinated movement of photoassimilates from leaves and other photoassimilating organs to places where they are consumed or stored.

Air pollutants, such as SO_2, O_3, NO_x, cause various changes in the translocation and distribution of metabolites (McLaughlin et al. 1983, Cooley and Manning 1987, 1988, Lechowicz 1987). Under the influence of

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Abbreviations: MES – morpholinoethanesulfonate, CCCP – carbonyl-cyanide-m-chlorophenyl hydrazone, K_i – inhibitor constant.

sulphur dioxide, an increase in the ratio of shoot and root biomass has been observed, mostly due to an increase in leaf biomass (Taylor et al. 1986, Lechowicz 1987, Mooney et al. 1988). Results of numerous studies show that the cause of these processes is the inhibition of translocation of photosynthates from sources to particular sinks (McLaughlin and McConathy 1983, Lorenc-Plucińska 1986, Taylor et al. 1986).

Experiments performed on various types of herbaceous plants show that inhibition of metabolite allocation results from direct damage to the phloem loading mechanism caused by SO_2 (Minchin and Gould 1986, Lorenc-Plucińska 1988, Maurousset and Bonnemain 1990).

The present study was aimed at determining the influence of SO_2 on the lateral transport of sucrose from the mesophyll to the conducting bundle tissue of tree leaves. These data have so far been absent in the literature with respect to trees. The experiments were performed on source leaf tissues of *Robinia pseudoacacia* L. This species was chosen for study because of its exceptional resistance to cold, drought, and soil pollution (Kowalkowski 1984, Rachwał and Kluczyński 1984, Łukasiewicz 1986).

MATERIAL AND METHODS

Fully expanded leaves from three-year-old locust (*Robinia pseudoacacia* L.) trees were used for all experiments. Leaves were collected in the morning, before the beginning of the light period and transferred with their petioles in H_2O to the laboratory. The leaves were washed in deionized water, the petioles were removed and the leaves were then immersed in water for 45 min to reduce day-to-day variability. Leaf discs (4 mm diameter) were excised from intercostal areas of non-abraded leaves, and transferred to a preincubation buffer (25 mM MES-NaOH (pH 6.0), 250 mM mannitol, 1 mM $CaCl_2$, 0.25 mM $MgCl_2$). They were vacuum infiltrated until most discs no longer floated on the medium. The discs were then floated for 2 h in a fresh buffer-solution to allow recovery from wounding and to deplete endogenous sugars.

Labelling of leaf discs with $^{14}CO_2$

Discs were preincubated for 30 min with illumination (54 nE/cm^2 · s) in a reaction mixture, containing 7 mM $NaHCO_3$, 250 mM mannitol, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$ and 25 mM MES-NaOH (pH 6.0) at 25°C. Fixation of $^{14}CO_2$ was initiated by adding $NaH^{14}CO_3$ (50 MBq/mmol) and sulfite, and was stopped after 30 min by transferring the discs to 6 N acetic acid. The leaf materials were digested and decolorized for 2 h in 0.25 mM 70% (v/v) perchloric acid plus 0.45 ml of 30% (v/v) H_2O_2 at 60°C and prepared for

scintillation counting (Beckman LS 5801). Sulfite was prepared with a MES-NaOH buffer.

Sucrose uptake

Studies of uptake kinetics were performed using the method of Lorenc-Plucińska and Ziegler (1989) with some modification. Briefly, the discs were preincubated for various lengths of time in a solution, containing 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 25 mM MES-NaOH (pH 6.0), 250 mM mannitol with or without CCCP (details are given in the legend to Table and Figures). Preincubation was followed by a 30 min uptake period in a solution, containing D-[U-^{14}C]sucrose (spec. act. 18.7 GBq/mmol) with or without various inhibitors at 25°C. After incubation, the discs were rinsed three times (3 min each) with unlabelled solution, decolorised and digested as above. The radioactivity was counted by liquid scintillation spectroscopy. All experiments were performed in the dark in a shaker bath (80 shakes min^{-1}).

Chemicals: labelled substances were purchased from Radiochemical Center Amersham; CCCP, sulfite, MES, mannitol from Sigma Chemical Co. (St. Louis, MO, USA); all other chemicals were analytical grade from POCh (Gliwice, Poland).

RESULTS

The processes of sugar transport in black locust are incompletely known. It has been established that the main component of phloem sap is sucrose (Zimmermann and Ziegler, 1975) and that sieve tubes contain different (depending on the season of the year) concentrations of adenine, ATP (Kluge and Ziegler 1964) and nucleic acids (Ziegler and Kluge, 1962). On this basis it has been assumed that the sugar transported from the mesophyll to phloem elements in black locust is sucrose and that the activity of phloem transport depends on the energy status of sieve tubes.

The uptake of sucrose into leaf discs of black locust does not increase linearly with solute concentration (Fig. 1). Two-component concentration profiles were generated in black locust discs: at lower sugar concentrations, a saturable component was operating, whereas beyond 25 mM sucrose a linear, diffusion-like component became apparent (Fig. 1).

The uptake from 20 mM [^{14}C]sucrose into isolated black locust discs was linear with time for about 30 minutes (Fig. 2). Under the influence of different exogenous sulfite concentrations the linear character of sugar uptake did not change with incubation time in the [^{14}C]sucrose medium. On the other hand,

there was a tendency towards a decrease in suger accumulation with an increase in the applied Na_2SO_3 (0.5–10.0 mM) concentrations. At these concentrations of sulfite, the greatest inhibition of sucrose uptake was observed during the first 5 min of the experiment (sulfite concentrations were not corrected during the experiments).

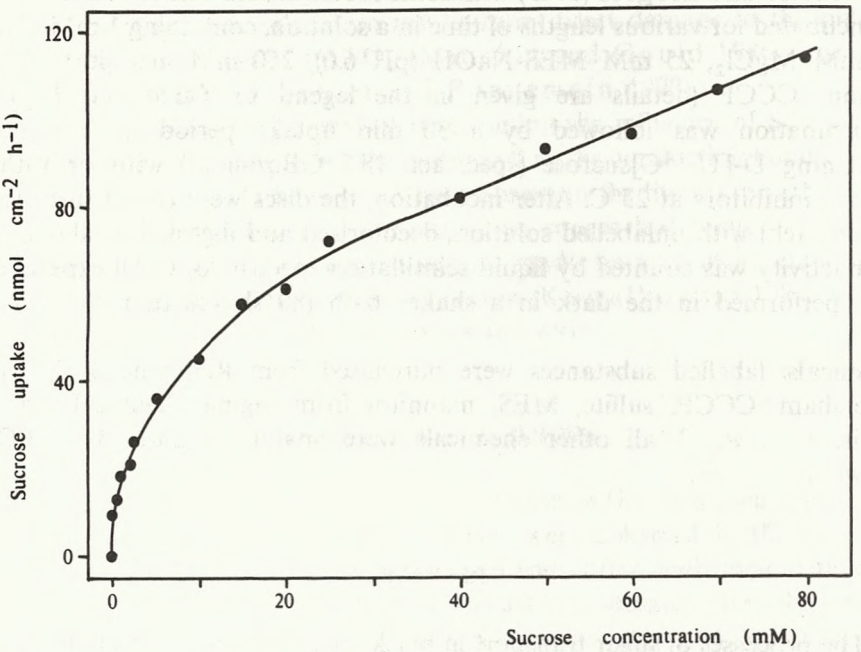

Fig. 1. Sucrose uptake by leaf discs at pH 6.0 in relation to dependence on sugar concentration. Points represent the mean for seven experiments (84 discs).

A statistically significant reduction in sucrose loading in the dark was noted already at 2.5 mM sulfite (Fig. 3). The assimilation of [$^{14}CO_2$] in light was reduced significantly only at a concentration of 10.0 mM sulfite (Fig. 3). The inhibition of sucrose accumulation by sulfite was of the non-competitive type (Fig. 4) and the calculated K_i was 6.3 mM of sulfite.

Uncouplers like CCCP change the proton electrochemical potential of the membrane directly. Sucrose uptake into tissue discs of black locust was severely inhibited by the presence of this uncoupler, as demonstrated in Table 1. While the control (without sulfite) tissue discs needed nearly 10^{-5}M CCCP for half-time inhibition, sucrose transport into leaf discs treated with 10 mM of sulfite was reduced by 60% in the presence of about 10^{-6}M CCCP. It should be noted, however, that sulfite itself caused a 54% inhibition of the process, compared to the control (Table 1).

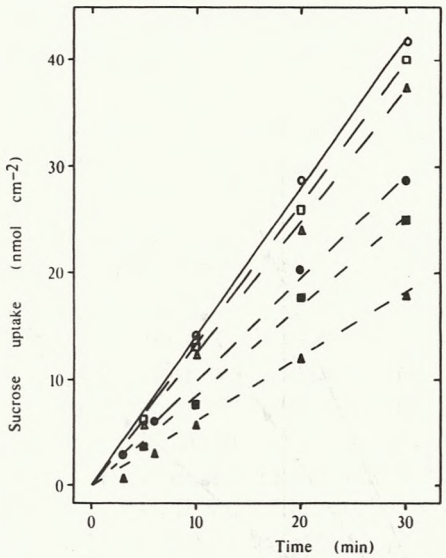

Fig. 2. The time-course kinetics of sucrose uptake (30 mM) in the presence of various concentrations of sulfite (o—o 0.0, □—□ 0.5, △—△ 1.0, ●—● 2.5, ■—■ 5.0, ▲—▲ 10.0 mM) at pH 5.5 Each value is the average of three independent experiments (36 discs).

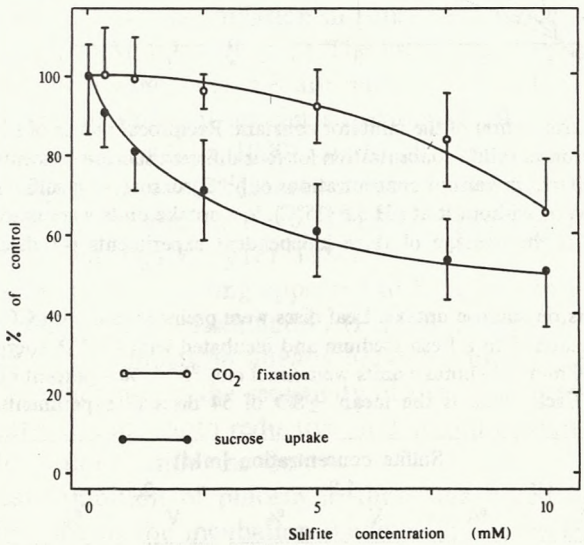

Fig. 3. Effect of sulfite on the degree of $^{14}CO_2$ fixation in light and [^{14}C]sucrose uptake in darkness by leaf discs. Incubation time of discs with sulfite was 30 min. Sulfite was added to the incubation medium simultaneously with 7 mM $NaH^{14}CO_2$ or 7 mM [^{14}C]sucrose. The rate of the processes in the control without sulfite is taken as 100%. Each point is an average ±SD from 4 sets (× 5 discs) in two experiments.

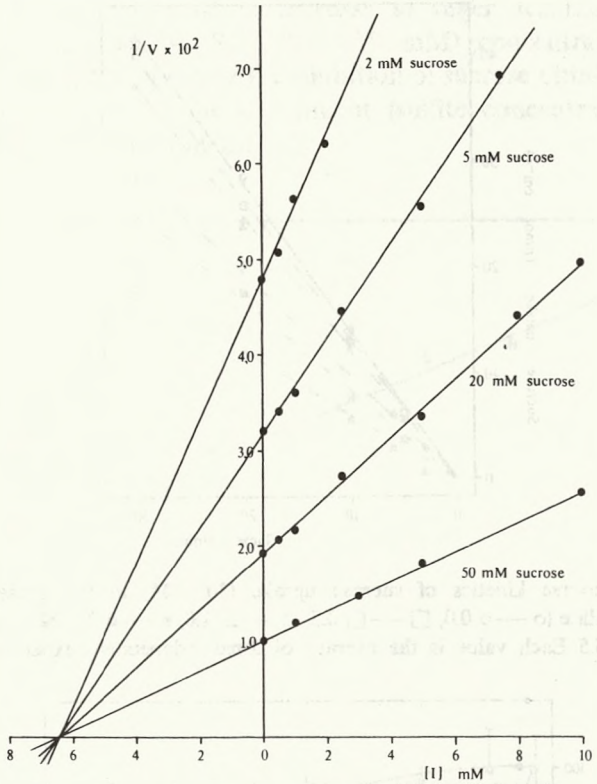

Fig. 4. Graphical determination of the inhibitor constant. Reciprocal values of sucrose uptake rate are plotted as a function of sulfite concentration for four different sucrose concentrations. The discs were incubated for 30 min in various concentrations of [^{14}C]sucrose with sulfite at concentrations from 0.25 to 10.0 mM or without it at pH 5.5 (25°C). V – uptake units were nmol cm^{-2}h^{-1}. Each value is the average of three independent experiments (48 discs).

Table 1

Influence of inhibitors on sucrose uptake. Leaf discs were preincubated with CCCP for 40 min at pH 6.0 and then transferred to a fresh medium and incubated with CCCP, sucrose (2.5 mM) and sulfite for a further 30 min. V – uptake units were nmol cm^{-2}h^{-1}. % – percent of control (without sulfite and CCCP). Each value is the mean ±SD of 54 discs (3 experiments).

	Sulfite concentration (mM)							
	0.0		1.0		2.5		10.0	
	V	%	V	%	V	%	V	%
nil	28±2.2	100	27±3.2	95	21±2	80	12±1.3	46
CCCP:								
100 μM	5.9±0.4	21	5.3±0.6	20	4.7±0.5	18	3.1±0.5	12
10 μM	14.6±1.6	52	14.3±1.1	51	13.2±1.8	47	8.2±1.2	29
1 μM	20.2±3.2	72	19.0±1.7	69	18.8±2.4	67	11.2±1.6	40

DISCUSSION

Robinia pseudoacacia has little value from a commercial point of view. It is, however, well known for its exceptional resistance to unfavourable influence of abiotic stress factors. It is recommended for post-erosive soils and industrially polluted areas. It the present investigation the exceptional resistance of black locust to SO_2 compared to other species of plants was manifested in its "reaction-response" to the effect of the inhibitor in the processes of photosynthesis and phloem loading.

In this study a buffer solution of sodium sulfite was used instead of SO_2. This procedure is often applied for two reasons. First, it is easier to control dose, pH and concentration when all reactions take place in one medium (water); and secondly, the results of the action of SO_2 and Na_2SO_3, respectively, are comparable, if not identical (Sakaki and Kondo 1985, Kozioł et al. 1986, Miszalski 1990). The pH values in all the experiments ranged from 5.5 to 6.0 According to Cape (1984), at pH 7.2 (characteristic for the cell cytoplasm) bisulfite and sulfite ions are present in the solution in equal proportions. In a more acidic environment the balance is shifted towards bisulfite (HSO_3^-), while with a more alkaline pH, sulfite ions (SO_3^{2-}) are dominant.

The intensity of $^{14}CO_2$ assimilation in source leaf tissues was significantly inhibited only at 10 mM sulfite (Fig. 3). The literature notes methodologically similar experiments in which a significant reduction of CO_2 assimilation in the light was observed in different species of plants at 10 times lower concentrations of sulfite (Miszalski 1990). In black locust no stimulation was observed with sulfite ions at concentrations below 1.0 mM, while this occurs in other plants (Ziegler and Libera 1975, Ziegler and Hampp 1977, Lorenc-Plucińska and Ziegler 1989).

The process of phloem loading appeared to be more sensitive to the action of sulfite ions than $^{14}CO_2$ assimilation (Fig. 3). A significant inhibition of exogenous sucrose accumulation already appeared under the influence of 2.5 mM sulfite. The difference in the sensitivity of these processes may have been caused by light-induced photo-reductive and photo-oxidative detoxification mechanisms for sulfite inside the tissues.

The greatest inhibition of phloem loading was noted immediately after applying sulfite ions to the incubation medium of source leaf tissues, and it decreased with incubation time. Because sulfite concentrations were not corrected during the experiments, it is difficult to say whether the effect results from lower sulfite concentration in the incubation medium, from its rapid oxidation to sulfate ions (less toxic!), from a change of activity in the investigated process or from lower sulfite uptake (as a defensive strategy).

The inhibition of sugar uptake by sulfite ions was of the non-competitive type (Fig. 4). Therefore, it can be assumed that sulfite does not affect the active center of the protein-carrier directly. The inhibition may be the result of structural changes in plasma membranes or the reaction between sulfite and SH-groups or S-S bonds of different proteins, which has already been described with respect to other plants (Lorenc-Plucińska 1988). The calculated K_i was 6.3 mM of sulfite (Fig. 4). Such a high K_i value indicates that at low inhibitor concentrations, the inhibition of sucrose loading will be insignificant, while at high concentrations, it may be complete i.e., it will cause a total blockage of phloem loading. It also indicates that even with low inhibition, the reaction rate will be high (see Fig. 2).

The metabolic uncoupler CCCP inhibits active sugar transport by limiting the ATP supply, eliminating the pH gradient on both sides of the membrane and depolarising the membrane potential (Giaquinta 1983). Sucrose uptake in leaf discs with and without sulfite was significantly inhibited by CCCP (Table 1). However, the reduction of phloem loading in the control tissue discs (without sulfite) and in the samples with sulfite at 0.1–2.5 mM was caused by inhibition of active sucrose uptake. On the other hand, in the samples treated with sulfite at a concentration of 10 mM, the above effect was accompanied by greater contribution of diffusion-like transport in the total amount of sucrose uptake (data not shown), which had been observed earlier with respect to other species of plants (Lorenc-Plucińska and Ziegler 1989).

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SUMMARY

The effect of sulfite (0.5–10.0 mM) on $^{14}CO_2$ fixation in light and on the uptake of exogenously supplied sucrose by source leaf tissues of *Robinia pseudoacacia* in the dark was studied. Sulfite at concentrations <10.0 mM did not significantly inhibit $^{14}CO_2$ assimilation. On the other hand, the rate of sucrose uptake was significantly inhibited at concentrations >2.5 mM. The inhibition of sucrose loading was of the non-competitive type and the calculated K_i was 6.30 mM of sulfite. Chemical modifier of the process of sucrose loading such as CCCP did not significantly affect the influence of sulfite at concentrations <10.0 mM and significantly increased the inhibition of this process by 10 mM sulfite. Sulfite at a concentration of 10.0 mM injured CCCP-sensitive and CCCP-insensitive uptake of sucrose.

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Zmiany w przemieszczaniu się cukru w liściach *Robinia pseudoacacia* L. pod wpływem SO_2

STRESZCZENIE

Badano wpływ dwutlenku siarki na natężenie asymilacji radioaktywnego dwutlenku węgla na świetle i pobierania egzogennie podanej radioaktywnej sacharozy przez izolowane krążki liściowe akacji białej.

W doświadczeniach zamiast SO_2 stosowano różne stężenia zbuforowanego roztworu siarczynu sodu, wychodząc z założenia, iż w środowisku wodnym wnętrza roślin, w zakresie pH typowych dla komórek roślinnych, pobierany z atmosfery dwutlenek siarki oddziaływuje na procesy metaboliczne w postaci jonów HSO_3^- i SO_3^{2-}.

Jony siarczynowe w stężeniach mniejszych niż 10 mM nie powodowały istotnych zmian w natężeniu fotosyntezy. Natomiast istotne obniżenie intensywności załadunku sacharozy obserwowano już pod wpływem stężenia siarczynu równego 2.5 mM (pH 5.5 lub 6.0, 25°C). Hamowanie to było typu niewspółzawodniczego, a stałą inhibicji K_i skalkulowano dla 6.3 mM siarczynu.

Zastosowanie siarczynu i CCCP wykazało, że pobieranie SO_3^{2-} w stężeniu <10.0 zaburza proces akumulacji sacharozy poprzez swój wpływ na aktywny transport cukru, a w stężeniu $\geqslant 10.0$ mM także na transport pasywny.