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The effects of root nitrogen supplies on the absorption of atmospheric NO_2 by soybean leaves

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SUMMARY

Twelve-day-old soybean plants were exposed to atmospheric NO_2 (0·3 μl l^{-1}) and simultaneously supplied, via the roots, with 5 mM or 1 mM of $NaNO_3$ or NH_4Cl. After exposure for 7 d, the amount of NO_2 absorbed per plant was greater in plants supplied with nitrate than in plants supplied with the same concentration of ammonium. The NO_2 AR (absorption rate) decreased with increasing exposure time. At the beginning of exposure, the NO_2 AR for all plants was *c.* 12 mg NO_2 h^{-1} m^{-2} μl^{-1} l. On the day 7 of exposure, the NO_2 AR declined to 8·46, 8·97, 8·27, and 9·04 mg NO_2 h^{-1} m^{-2} μl^{-1} l for plants receiving 1 mM ammonium, 1 mM nitrate, 5 mM ammonium, and 5 mM nitrate respectively. The plants supplied with nitrate had a higher concentration of leaf nitrate and a higher pH than those supplied with the equivalent concentration of ammonium. These results suggest that the NO_2 absorption rate might be attenuated by the accumulation of H^+ produced from N uptake and assimilation.

Key words: Absorption of nitrogen dioxide, nitrogen supply, soybean.

INTRODUCTION

There have been several studies concerning the effects of root N supply on NO_2 uptake by leaves. Bush bean plants grown with urea as the nitrogen source had a higher NO_2 absorption rate (AR) than those given nitrate as the nitrogen source. Deficiency in nitrate supply also affected NO_2 uptake. A higher AR occurred in N-starved bean leaves (Srivastava, Jollifee & Runeckles, 1975). By contrast, however, the rate of NO_2 flux into the leaves of N-deficient barley was lower than that into N-sufficient plants (Rowland-Bamford & Drew, 1988). In sunflower plants exposed to 2 μl l^{-1} NO_2 the AR was lower in plants grown at a lower concentration of nitrate (Okano & Totsuka, 1986), but in corn and soybean plants, the concentration of tissue N did not influence NO_2 AR (Rogers, Jeffries & Witherspoon, 1979). In order to understand better the mechanism of NO_2 absorption, more information about the relationship between NO_2 absorption and root N supply is required.

Okano, Machida & Totsuka (1988) measured the NO_2 AR, and its response to NO_2 exposure, in eight herbaceous species, finding that plants with a higher AR were more susceptible to NO_2 than those with a lower NO_2 AR. Bean plants supplied with different forms of root N (nitrate or ammonium) showed different foliar injuries by NO_2. Ammonium in the nutrient solution appeared to augment NO_2 injury in leaves (Srivastava, Ormrod & Hale, 1992)

The main pathway for NO_2 absorption by leaves is considered to be diffusion through the stomata followed by dissolution in the extracellular fluid (Okano *et al.*, 1988; Okano, Machida & Totsuka, 1989; Wellburn, 1990). The factors that influence diffusion of NO_2 into the stomata have been discussed in previous studies (Okano *et al.*, 1988; Rowland-Bamford & Drew, 1988; Hanson & Lindberg, 1991). However, the effects of the chemical components of extracellular fluid on NO_2 absorption have been investigated to a lesser extent. Since the products of NO_2 dissolution in extracellular fluid are H^+, nitrate and nitrite (Wellburn, 1990; Bambauer *et al.*, 1994) which would retard further dissolution of NO_2, we postulated that the increase in leaf concentration of those products might inhibit NO_2 absorption by leaves.

MATERIALS AND METHODS

Growth of plants

Soybean seeds (*Glycine max* L. Oxley) were germinated in tissue rolls, and twenty healthy seedlings

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were chosen for hydroponic culture. Each seedling was planted in a clay-pellet substrate in a plastic strawberry pot (50 mm high, 40 mm diameter at the base, 58 mm diameter at the top, volume 90 ml). Each pot was placed in one of 20 holes (50 mm in diameter) in a hard plastic plate (420 × 320 × 5 mm), which served as the lid of a plastic tray (400 × 300 × 130 mm) containing 10 l of nutrient solution. The root tips of the seedlings were immersed in the nutrient solution which was aerated by an air pump. The solution composition was (g m^{-3}): $CaCl_2$ 222, KH_2PO_4 68, $K_2HPO_4.{}_3H_2O$ 114, K_2SO_4 522, $MgSO_4$ 240, NH_4NO_3 405, FeEDTA 36, H_3BO_3 2·86, $MnSO_4.H_2O$ 0·563, $ZnSO_4.7H_2O$ 0·44, $CuSO_4.5H_2O$ 0·15, $CoCl_2$ 0·10, $NaMoO_4.2H_2O$ 0·05. The pH was controlled at 6·2 ± 0·2 by the addition of H_2SO_4 or NaOH. The solution level in the tray was kept constant by adding fresh solution as required. Twelve days after germination, six healthy seedlings of similar leaf development were selected for NO_2 fumigation.

Fumigation

Plants for fumigation were suspended in individual nutrient containers with an internal volume of 1·4 l. Each container was aerated with filtered air at a flow rate of 30 ml per minute through a 22 gauge hypodermic needle. A glass side arm with index mark and plugged with cotton wool was used to indicate nutrient level and a Subaseal® located on the side of the container allowed sampling of the solution or topping up when required.

Each plant was then enclosed in a rectangular glass cuvette 200 × 200 × 380 mm with a detachable, split base isolating the plant top from the roots. Each cuvette contained a 40 mm fan to assist mixing, and a temperature probe. An inlet and outlet were located at diagonally opposite corners of the cuvette. The plant was sealed to the base with Blutak (Bostik, Thomastown, Australia) and the base sealed to the nutrient container with tape to prevent gas exchange between the cuvette and the container.

The cuvettes were placed in a constant temperature room (303 ± 1 K) and illuminated by a timer-controlled 1000 W metal halide lamp directed through a water filter designed to minimize temperature effects. Each cuvette inlet was connected to a flow meter delivering filtered air at 1 l min^{-1}. Flow meters delivering NO_2 were connected to a mixing chamber into which NO_2 was introduced by a mass flow control valve model 5850 TR (Brooks Instrument Div., Emerson Electric Company, Hatfield). Thus every cuvette received the same concentration of NO_2 at its inlet. The outlet was connected to a two-way solenoid valve allowing the gas either to be vented to the atmosphere or directed to a 8840 nitrogen oxides analyser (Monitor Labs, San Diego) with a flow rate of 500 ml min^{-1}. An identical, but empty, cuvette was used to monitor adsorption of fumigation gases onto the surfaces of the cuvette and extra solenoid valves allowed the inlet gases to be diverted to the analyser. All plumbing materials used in the study were either glass or Teflon®.

Fumigation was controlled by an electronic timer which switched individual valves in sequence every 10 min throughout the fumigation period. Data were logged on a model 7000B macro data logger (Unidata, Perth) which included cuvette number, temperature, PAR (photosynthetically active radiation) and nitrogen oxides.

The initial concentration of fumigating NO_2 was 0.3 μl l^{-1}. For each batch of plants, fumigation lasted for 7 d, with illumination for 12 h d^{-1} synchronized with fumigation from 0600 to 1800 hours. The average PAR was 320 μmol m^{-2} s^{-1} during daytime. On days 3 and 5 of fumigation, *c.* 50 ml of fresh solution was added to each container with a syringe to compensate for evaporation and uptake of the solution. There were four batches of plants fumigated for this experiment.

Measurement and analysis

The leaf area for each plant on day 7 of exposure was measured with a photometric area-meter. Leaves were rinsed with deionized water and blotted with tissues. Each leaf was cut in half. One half of the leaves from each plant was weighed, cut into small pieces, ground in a mortar with a pestle, and mixed with deionized water weighing 20 times that of the leaves. The pH of the slurry was measured. The other half of the leaves from each plant was dried in a forced draught oven for 65 h at 70 °C. The dry leaves were ground into a fine powder for determination of nitrate, nitrite and Kjeldahl nitrogen (Singh, 1988; Srivastava, Ormrod & Hale, 1994).

NO_2 AR was calculated using formula (1):

$$\mathrm{AR} = \frac{1{\cdot}85 \times 10^{-3} F(C_{\mathrm{in}} - C_{\mathrm{out}})}{0.5(C_{\mathrm{in}} + C_{\mathrm{out}})A} \qquad (1)$$

where AR = NO_2 absorption rate (mg NO_2 h^{-1} m^{-2} μl^{-1} l). $1{\cdot}85 \times 10^{-3}$ = the mass (mg) of 1 μl of NO_2, F = the flow rate of the fumigation mixture flowing through a cuvette (60 l h^{-1}), C_{in} = NO_2 concentration at the outlet of the empty fumigation cuvette (0·3 μl l^{-1}) (this equalled the NO_2 concentration at the inlets of the cuvettes if NO_2 adsorption by cuvette walls was ignored), C_{out} = NO_2 concentration at the outlet of a cuvette containing a fumigated plant (μl l^{-1}), $0{\cdot}5(C_{\mathrm{in}} + C_{\mathrm{out}})$ = the average NO_2 concentration in a cuvette (μl l^{-1}) and A = leaf area of a plant (m^2).

As absorption of NO_2 increases linearly with increasing concentration (Okano *et al.*, Kukuzawa, Tazaki & Totsuka, 1986; Segschneider, Jurgen &

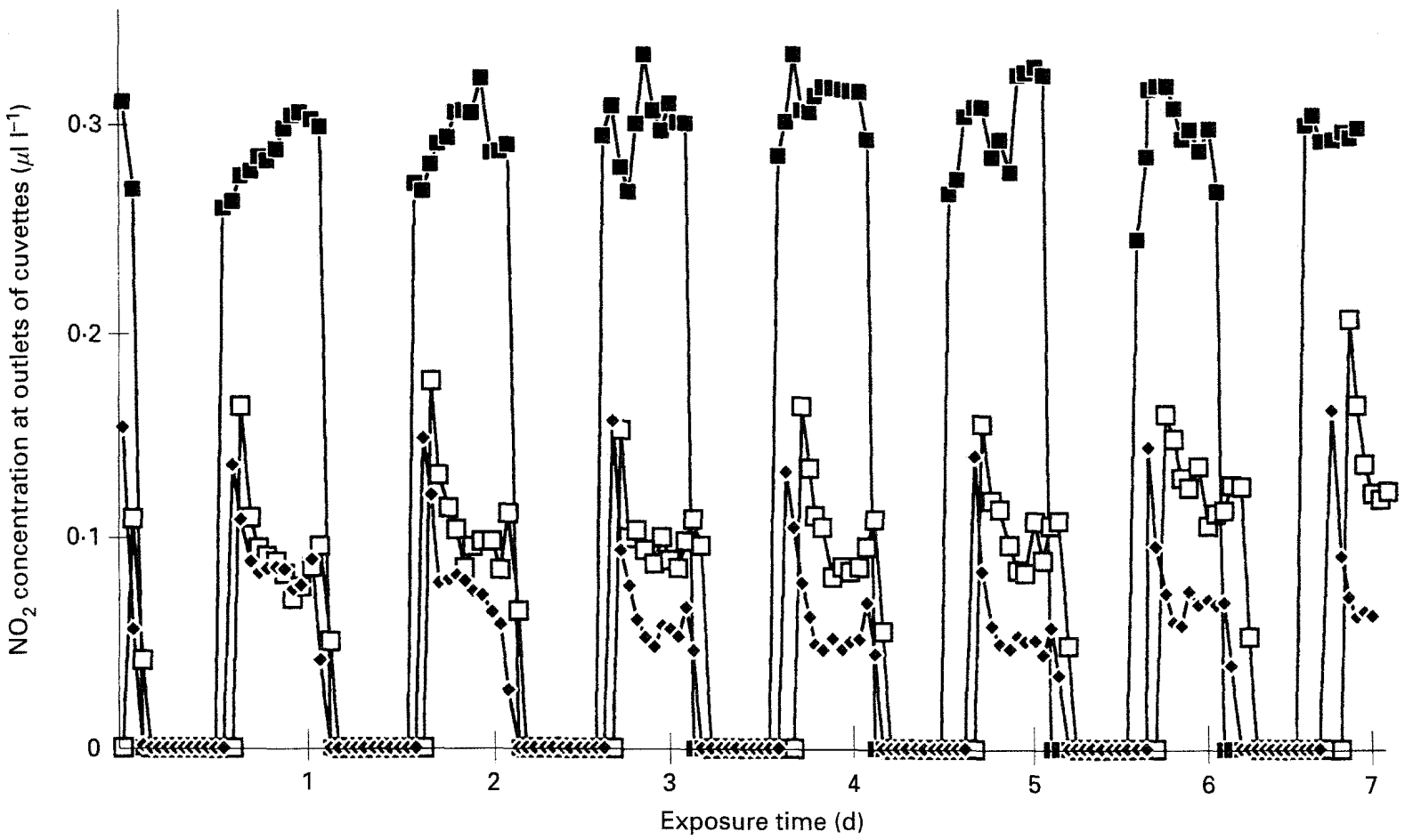

Figure 1. Effect of form of root N supply on NO_2 absorption. The concentration of NO_2 was measured at the outlet of an empty cuvette (■), a cuvette containing a plant receiving 5 mM ammonium (□), and a cuvette containing a plant receiving 5 mM nitrate (◆). The initial concentration of NO_2 was 0·3 μl l^{-1}, given only during the period of illumination. For further details, see the 'Materials and Methods' section.

Table 1. *Mean squares from the two-way ANOVA for the effects of root N supplies on NO_2 AR, leaf NO_3^--N, and leaf pH*

Source of variation	d.f.	NO_2 AR ($\times 10^2$)	Leaf NO_3^--N	Leaf pH ($\times 10^4$)
Concentration of root N	1	2·312	19·37***	57·04**
Species of root N	1	202·2*	36·26***	3528***
Species × concentration of root N	1	6·962	15·75***	315·4***
Error	16† 20‡	35·46	0·1082	3·4083

Levels of significance: $*P < 0·05$; $**P < 0·01$; $***P < 0·001$.
† For NO_2 AR.
‡ NO_3^- and pH.

Forstel, 1995), comparison of the ability of plants to absorb NO_2 needs to be on a basis of equal NO_2 concentration. Although the NO_2 concentration at the inlet of every cuvette was the same in this experiment, the concentrations at the outlets were different at the end of exposure (Fig. 1). We therefore used the average concentration, $0·5(C_{in}+C_{out})$, to represent the practical concentration in each cuvette for calculation of NO_2 AR. Thus the value of NO_2 AR used in this work (mg NO_2 h^{-1} m^{-2} μl^{-1} l) refers to the amount of NO_2 (mg) absorbed by 1 m^2 area of leaf exposed to 1 μl l^{-1} NO_2 for 1 h.

Two-way ANOVA and Duncan's New Multiple Range Test were used to test the differences in NO_2 AR, leaf nitrate concentration, and leaf pH among the different treatments (Beyer, 1974; Ott, 1977) (Table 1).

RESULTS

NO_2 absorption

At the beginning of fumigation, each cuvette containing a plant showed similar NO_2 concentration at the outlets (Fig. 1). As plants of similar leaf development were selected for exposure, they had almost the same initial leaf area, so they had almost the same initial NO_2 AR. Using the approximate

Table 2. *NO_2 absorption rate (mg NO_2 h^{-1} m^{-2} μl^{-1} l) for soybean plants supplies with different forms of root N on the seventh day of exposure to 0·3 μl l^{-1} NO_2*

Concentration of root N supply	NO_2 absorption rate	
	Ammonium	Nitrate
1 mM	8·46 ± 0·59 ab	8·97 ± 0·57 ab
5 mM	8·27 ± 0·64 a	9·04 ± 0·59 b

Each value represents the mean ± SD for five plants in each treatment.

Means not followed by the same letter are significantly different at $P < 0·1$.

Table 3. *Leaf nitrate-N concentration (mg NO_3^--N g^{-1} d. wt) for soybean plants supplied with different forms of root N and exposed to 0·3 μl l^{-1} NO_2 for 7 d*

Concentration of root N supply	Leaf nitrate-N concentration	
	Ammonium	Nitrate
1 mM	1·56 ± 0·32 a	2·40 ± 0·38 b
5 mM	1·74 ± 0·24 ab	5·82 ± 0·36 c

Each value represents the mean ± SD for six plants in each treatment.

Means not followed by the same letter are significantly different at $P < 0·01$.

average initial leaf area, the initial NO_2 AR was computed to be *c.* 12 mg NO_2 h^{-1} m^{-2} μl^{-1} l. After exposure for 7 d, the amount of NO_2 absorbed per plant was noticeably lower for plants grown with N supplied as ammonium than that for plants given the same concentration of N supplied as nitrate (Fig.1). However, only at 5 mM was this difference significant at the level $P < 0.1$ (Beyer, 1974; Ott, 1977; Moore & McCake, 1993). No differences in NO_2 AR among the other treatments were significant (Tables 1, 2).

Leaf nitrate and nitrite

The concentrations of nitrate in the leaves of nitrate-supplied plants were higher than those in ammonium-supplied plants ($P < 0.01$). Similarly, plants at 5 mM nitrate had higher leaf nitrate concentrations than those at 1 mM nitrate ($P < 0.01$) (Table 3).

The concentrations of nitrite in the leaves were very low and there were no significant differences among the different treatments.

Leaf pH

The leaf pH was higher in plants supplied with nitrate than in those given the same concentration of ammonium ($P < 0.01$). The maximum pH difference occurred between plants fed with 5 mM nitrate and ammonium (Table 4).

Table 4. *Leaf pH for soybean plants supplied with different forms of root N and exposed to 0·3 μl l^{-1} NO_2 for 7 d*

Concentration of root N supply	Leaf pH	
	Ammonium	Nitrate
1 mM	6·35 ± 0·02 a	6·52 ± 0·015
5 mM	6·31 ± 0·02 a	6·62 ± 0·02 b

Each value represents the mean ± SD for six plants in each treatment.

Means not followed by the same letter are significantly different at $P < 0·01$.

DISCUSSION

Nitrogen-deficiency is known to decrease stomatal opening and increase leaf resistance (Ryle & Hesketh, 1969; Rowland-Bamford & Drew, 1988). It might therefore cause a decrease in NO_2 AR. However, in our experiment, the plants grown at 5 mM ammonium did not show any symptoms of N-deficiency. This corresponds with the finding of Rogers *et al.* (1979), using soybean and corn plants. The concentration of leaf N at which soybean plants are considered N deficient is 2·2–3·2 % (Reuter & Robinson, 1986). In the leaves of plants supplied with 5 mM ammonium in our experiments, the Kjeldahl N concentration was 4–6 %. Therefore, N-deficiency was not the main reason for the observed decrease in NO_2 AR for the plants at 5 mM ammonium.

Söderlund (1981) and Wellburn (1990) indicated that the pH value and the presence of solutes in extracellular fluid might affect NO_2 absorption by leaves. Both the NO_2 AR and the pH of the leaves of plants receiving 5 mM ammonium were lower than those for plants given 5 mM nitrate (Tables 2, 4). This suggests that increase in H^+ in leaves might inhibit NO_2 absorption.

The reaction of dissolution of NO_2 in water (Wellburn, 1990; Bambauer *et al.*, 1994), $2NO_2 + H_2O = 2H^+ + NO_3^- + NO_2^-$, shows that H^+ exerts a predominant effect over nitrate on NO_2 dissolution. Because assimilation of ammonium produces H^+ and assimilation of nitrate consumes H^+ (Raven & Smith, 1976), the H^+ concentration in leaves of the plants at 5 mM nitrate was half that for the plants at 5 mM ammonium (Table 4). This difference in H^+ concentrations of leaves might reflect a difference in the H^+ concentrations of extracellular fluid, which in turn would decisively affect NO_2 absorption, even though there is also a small opposite effect due to the different nitrate concentrations in ammomium-grown over nitrate-grown plants (Table 3).

The plant leaves grew quickly during exposure to NO_2 (the leaf area for each plant doubled during the 7 d period), but the NO_2 concentration at the outlets

(Cout) of the cuvettes containing plants given ammonium increased rather than decreased with increasing leaf area (Fig.1). This indicates that the NO_2 AR declined with increasing exposure time. In plants supplied with 5 mM or 1 mM ammonium, the NO_2 AR declined from 12 units at the beginning of exposure to 8·27 or 8·46 units on the day 7 of exposure (Table 2). This might result from accumulation of H^+ produced by NO_2 absorption (Wellburn, 1990; Bambauer *et al.*, 1994) and from ammonium assimilation (Raven & Smith, 1976) in the plants supplied with ammonium as the only root N source. As assimilation of nitrate can consume H^+ produced from NO_2 absorption, the NO_2 AR of the plants grown with nitrate declined more slowly than in plants grown with ammonium during exposure (Table 2; Fig 1).

This decline in NO_2 AR with increasing exposure time, which is analogous to a decline in concentration of fumigating NO_2, might explain the acclimation of plants to long-term NO_2 exposure (Hufton, Besford & Wellburn, 1996). Thus, low concentrations of NO_2 often have a stimulatory effect on plants, in marked contrast to the inhibitory effect of high concentrations of NO_2.

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