Table 1. The effect of methylglyoxal Bis(guanylhydrazone) (MGBG) with and without putrescine, spermidine, or spermine on *Dugesia tigrina* auricle regeneration

Treatment	Number of animals	Mean time for auricle regeneration ± SD (days)
Control	20	6.20 ± 0.83
Putrescine (1×10^{-4} M)	20	$5.40 \pm 0.72^{a,b}$
Spermidine (1×10^{-4} M)	20	$5.05 \pm 0.67^{a,b}$
Spermine (1×10^{-4} M)	20	5.70 ± 0.58^{b}
MGBG (5×10^{-4} M)	17	8.85 ± 1.61^{a}
Putrescine (1×10^{-4} M) + MGBG (5×10^{-4} M)	17	$6.80 \pm 0.90^{a,b}$
Spermidine (1×10^{-4} M) + MGBG (5×10^{-4} M)	20	5.95 ± 1.05^{b}
Spermine (1×10^{-4} M) + MGBG (5×10^{-4} M)	18	6.00 ± 0.76^{b}

[a] $p < 0.01$ vs control; [b] $p < 0.01$ vs MGBG (5×10^{-4} M) treatment.

Table 2. The effect of α-methylornithine (α-MO) with and without putrescine, spermidine, and spermine on *Dugesia tigrina* auricle regeneration

Treatment	Number of animals	Mean time for auricle regeneration ± SD (days)
Control	15	6.33 ± 1.08
Putrescine (1×10^{-4} M)	15	$5.13 \pm 0.35^{a,b}$
Spermidine (1×10^{-4} M)	15	$4.93 \pm 0.25^{a,b}$
Spermine (1×10^{-4} M)	15	$5.00 \pm 0.00^{a,b}$
α-MO (5×10^{-4} M)	15	8.60 ± 1.12^{a}
Putrescine (1×10^{-4} M) + α-MO (5×10^{-4} M)	15	$5.06 \pm 0.25^{a,b}$
Spermidine (1×10^{-4} M) + α-MO (5×10^{-4} M)	14	$5.21 \pm 0.50^{a,b}$
Spermine (1×10^{-4} M) + α-MO (5×10^{-4} M)	15	6.32 ± 0.42^{b}

[a] $p < 0.01$ vs control; [b] $p < 0.01$ vs α-MO (5×10^{-4} M) treatment.

midine, or spermine at a concentration of 1×10^{-4} M), an inhibitor (MGBG or α-MO at a concentration of 5×10^{-4} M), or one of the amines (1×10^{-4} M) plus one of the inhibitors (5×10^{-4} M). The animals were then observed daily for the reappearance of auricles.

Results. The results are summarized in tables 1 and 2. Both inhibitors, MGBG and α-MO, significantly increased the mean time needed for auricle regeneration (analysis of variance and Tukey's (a) test were used). In both experiments, each amine when used alone reduced the mean time needed for auricle regeneration and each was effective in preventing or reducing the regeneration retardation effect of both inhibitors.

Discussion. Rupniak and Paul[6] using MGBG arrested the growth of cultured rat embryo fibroblasts and upon adding spermidine or spermine observed a rapid resumption of cell proliferation. Using α-MO, Mamont et al.[4] inhibited proliferation of rat hepatoma cells in culture. Addition of putrescine, spermidine, or spermine resulted in an immediate resumption of cell proliferation. Our results parallel these reports and further support the idea that the amines studied are needed for cell proliferation processes in general.

Flatworms have been neglected as subjects of polyamine research. Yet they are inexpensive, easily maintained and manipulated in the laboratory, and exhibit a spectacular ability to regenerate. The authors hope that this report and the one by Forbes et al[1] will stimulate an interest in utilizing flatworms in studies of the role of polyamines in growth processes.

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Uptake of NO_2 by plants grown at different salinity levels

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Summary. It is demonstrated that the uptake of nitrogen dioxide (NO_2) by *Phaseolus vulgaris* L. is decreased by the addition of sodium chloride (NaCl) to the root medium, as a result of increased diffusive resistance of the leaves. The NO_2-uptake rate constant measured kinetically was in agreement with the nitrite content of the leaves after the fumigation.

The uptake of gaseous pollutants by plants is a well known fact. Knowledge about the absorption of air pollutants under various environmental conditions is important with regard to the function of the plants as a sink. Several studies were carried out to demonstrate how the rate of absorption is related to the stomatal resistance[1,2]. It is generally accepted that stomatal resistance is inversely proportional to the molecular diffusivity of the gas of interest. The stomatal resistance of the leaves depends on such factors as the type, age and condition of the plant; the latter aspect is influenced by the water availability, solar radiation level, air temperature, concentration of CO_2 in the atmosphere, etc., as well as soil properties and damage by pollutants[3]. Under controlled environmental conditions the uptake of NO_2 has been thoroughly studied from a chemical kinetic viewpoint[4]. Under natural conditions, however, plants are rarely exposed to a single pollutant. In the severely polluted roadside environments of northern countries several gaseous pollutants deriving from automobile exhausts influence the plants, together with sodium chloride used as de-icing salt. Excessive soil salt may affect the health of plants by inducing osmotic stress, via specific ion toxicity, through changes of pH, by creating a nutrient imbalance or by changing soil permeability or structure[5]. The relevance of the combined effect of salt and gaseous pollutants prompted us to demonstrate the influence of different salinity levels in the root medium on the uptake of NO_2 by leaves of *Phaseolus vulgaris* L. Seeds of *Phaseolus*

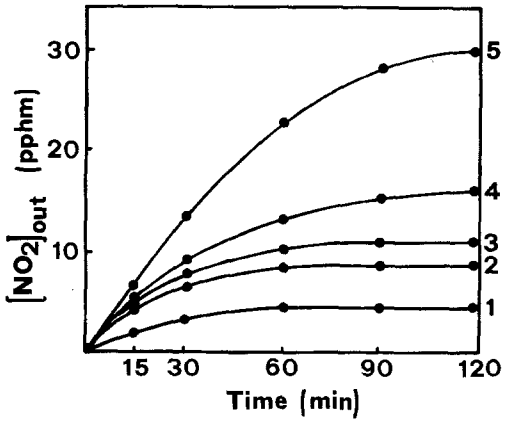
NO_2-uptake rate constant, leaf diffusion resistance and nitrite content of the leaves of 14-day-old bean plants grown at different salinity levels and fumigated with 31 pphm NO_2 for 2 h

NaCl concentration (mM)	NO_2-uptake rate constant ($\mu g \cdot dm^{-2} \cdot min^{-1} \cdot pphm^{-1}$)	Leaf diffusion resistance* ($sec \cdot cm^{-1}$)	Nitrite content** ($\mu moles \cdot ml^{-1}$ sap)
0	8.7	3.8±0.5	10.2±2.1
20	3.2	6.3±1.3	5.3±1.5
40	2.2	10.1±2.5	4.0±1.3
80	1.0	19.2±4.1	2.7±1.0

* Measured before fumigation. Mean value for 6 plants (± SEM). ** Measured after fumigation. Mean value for 6 plants (± SEM).

vulgaris L. var. *Saxa (Radio) Stamm Vatter* (bush bean) were germinated on wet tissue paper (3 days) and on wet quartz sand (2 days in darkness and 2 days in light). The plants were then cultivated in a plastic tank containing nutrient solution at a day temperature of 25 °C and a night temperature of 21 °C under 8 klux during 14 h a day. Relative humidity ranged from 70% (day) to 80% (night). Nutrient solution contained, per 1 distilled water: 0.3 g K_2HPO_4, 0.4 g KH_2PO_4, 0.75 g $MgSO_4 \cdot 7\ H_2O$, 0.042 g FeEDTA, 0.89 g $CaSO_4$, 0.236 g $(NH_4)_2SO_4$ and 0.5 ml trace element solution (containing per 1:0,39 g $MnCl_2 \cdot 4\ H_2O$, 0.61 g H_3BO_3, 0.098 g $ZnSO_4 \cdot 7\ H_2O$, 0.055 g $CuSO_4 \cdot 5\ H_2O$, 0.06 g MoO_3) Salinities were imposed by adding various amounts of NaCl giving a range of concentrations from 20 to 80 mM. Plants were adapted to the salinity during 3 days. Fumigation with NO_2 was carried out under the same environmental conditions with 14-day-old plants in a PVC fumigation chamber (610 · 410 · 355 mm). Fumigation time was 2 h. NO_2 from a tube with N_2O_4 was injected into the air stream flowing through the chamber at a rate of 40 l/h. The inlet concentration was 0.31 ppm. The NO_2-concentration was measured by passing an air sample through the Griess-Saltzman reagent[6]. The diffusive resistance of the leaves was measured before starting the fumigation (1 h after turning on the light) using a modification of the diffusion porometer described by Körner and Cernusca[7]. Immediately after fumigation leaves were harvested and extracted with 0.1 N NaOH. Protein was removed by precipitation with $BaCl_2$[8]. After centrifugation of the protein, nitrite was estimated according to Sanderson and Cooking[9]. The NO_2-uptake rate was calculated from the inlet and outlet concentrations as described by Rogers et al.[4].

The figure shows the results of 5 different fumigation experiments. Plotted are the time-courses of the NO_2-concentration at the chamber outlet with 12 plants grown at one of the following salinity levels: (1) 0 mM; (2) 20 mM; (3) 40 mM; (4) 80 mM NaCl. Plot No. 5 shows the outlet of the empty chamber. A small loss of NO_2 due to chamber wall losses was inevitable. 2 h after the beginning of the fumigation the outlet concentration remained constant. Since at this moment the concentration in the chamber as well as the plant surface area and the NO_2 loss rate were constant, a rate constant for the uptake could be estimated, corresponding to each salinity level used. The results are given in the table, together with diffusive resistances and nitrite contents of the leaves after the fumigation period.



Nitrogen dioxide concentrations at the chamber outlet with 12 14-day-old bean plants with a total leaf area of 11.5±0.5 dm^2, grown at different salinity levels. *1* 0 mM, *2* 20 mM, *3* 40 mM, *4* 80 mM sodium chloride (NaCl), *5* outlet of the empty chamber. Inlet concentration was 31 pphm.

It is evident that NO_2-uptake is reduced when the plants are grown at increasing salinity levels. This must be due to the increasing diffusive resistance of the leaves, which in turn depends from the water potential of the root medium[10].

The uptake rate constant as calculated from the kinetics shown in the figure is in agreement with the nitrite content of the leaves after the fumigation period. During fumigation with NO_2 both nitrite and nitrate are formed in the leaves[8]. With regard to the naturally growing plants in polluted areas (e.g. roadside) the important point of our results is that the absorption capacity for gaseous pollutants is reduced by salt in the root medium. Therefore the function of the vegetation as a sink for gaseous pollutants is reduced.

On the other hand NO_2 is proved to be a damaging agent, although little is known about the process leading to visible plant injury. The exclusion of NO_2 or similar gases by closure of the stomata could therefore be considered as a protective measure.

This possible antagonism could be confirmed by field experiments. Beans exposed in areas with high level air pollution were less severely injured when they were grown at saline substrates. According to the stress terminology this enhancement of the resistance to air pollution stress is due to avoidance of the stress by exclusion of the damaging agent[11].

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