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ALLOCATION OF BIOMASS, NITROGEN, AND SULPHUR IN ACID MIST TREATED PLANTS

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Abstract. Plants of *Phaseolus vulgaris* L. were exposed to mist consisting of distilled water at pH 5.6 (control) or $H_2SO_4+HNO_3$ (molar ratio 1.5:1) in distilled water at pH 4.5, 3.0, and 2.3. Nitrogen and sulphur displayed different patterns among the various plant organs. A marked increase in sulphur and nitrogen in plants exposed to elevated levels of SO_4^{2-} and NO_3^- in the treatment solution was observed. The highest content of nitrogen was measured in the leaves, that of sulphur in the roots. No essential changes in height, but significant reduction of root biomass and disbalance of the ratios above-ground organs/roots, nitrogen/dry mass, and sulphur/dry mass in different organs were found in pH 2.3 acid mist treated plants.

Key words: acid mist, *Phaseolus vulgaris* L., nitrogen, sulphur, leaf injury, biomass.

INTRODUCTION

The effect of acid rain on plants has been discussed in several review articles (Likens & Jonson, 1972; Proctor, 1983; Neufeld et al., 1985; Nygren & Hari, 1992). Responses of plant growth are often species-specific. Some plants show enhanced growth (Wood & Borman, 1974), some no response at all (Thomas et al., 1952; Lee et al., 1981), and others reduced growth (Neufeld et al., 1985). The mechanisms responsible for the growth changes due to acid rain in modelled experiments or in natural conditions are not yet completely understood. Changes in the physiology and biochemistry of plants, alterations in the photosynthesis and respiration, mineral nutrition, and translocation rates of assimilates or nutrients have been suggested to explain the growth and biomass formation of plants under the acid rain application (Ferenbaugh, 1976; Hindawi et al., 1980; Neufeld et al., 1985).

The purpose of the present study was to ascertain the effect of acid precipitation on the content and allocation of mineral nutrients and on the biomass in *Phaseolus vulgaris* L. and to detect changes in its different organs during prolonged acidic exposure depending on the pH of treatment.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L. cv. Valja were germinated in plastic pots filled with fertilized peat in a greenhouse (two plants per pot). Then the plants were kept at 22±2 °C at an average light intensity of 250—400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (measured with Yanishevsky's pyranometer) in the daytime, and relative humidity of 70—80%. At the beginning of the experiment the plants with well-developed primary leaves with the central leaflet of the first trifoliate leaf were selected and distributed equally between four growth chambers (1 m^3), 43 plants in each chamber. During the experiment the plants were treated using the spraying system Englo with different pH values (2.3, 3.0, 4.5) of $H_2SO_4+HNO_3$ (molar ratio 1.5 : 1) solutions. The control plants were sprayed with distilled water of pH 5.6. Plants were subjected to simulated acid mist of 50 $ml \cdot m^{-2}$ at a frequency of one treatment per day. The leaves were thoroughly wetted but only a small amount of the solution that ran off the leaves fell onto the base of plants. The duration of the experiment was 21 days. The character of the nutrient elements assimilable by the plants in the growth substrate at the beginning and the completion of the experiment is shown in Table 1.

Table 1

Chemical character of the growth substrate of experimental plants

Treatment pH	pH_{KCl}	Total N, %	P_2O_5	K_2O	Ca	Mg	Cu	Zn	Fe	Mn	S
			mg · 100 g⁻¹				mg · kg⁻¹				
Initial content											
	5.40	0.89	50	150	960	90	3.0	14.0	290	24	960
At the end of the experiment											
5.6	4.80	0.79	24	24	700	60	2.0	9.5	370	13	660
4.5	4.78	0.78	13	15	680	50	3.0	10.5	340	12	690
3.0	4.90	0.75	12	17	760	60	3.0	11.6	370	13	870
2.3	4.90	0.87	12	17	730	60	3.0	10.5	380	15	870

During the experiment the height of the plants was measured, and the appearance of all visible injuries on leaves at different treatment levels was registered. In the end of the experiment injuries on the upper surface of every leaf of each plant were assessed visually by placing a transparent grid on the leaves. The percentage of leaf area injured was defined as the number of grid intersections covering injured areas divided by the total number of intersections on the leaf. This method was recommended by Cumpertz et al. (1982) and we found it to be the most suitable for estimating visible injuries on leaves.

After a 21-days' experiment the roots, stems, and leaves of the plants were separated for biochemical analyses, carefully washed, and dried at 105 °C. To detect mineral elements average samples of ten plants were used. These were analysed in three replications. Nitrogen was analysed using Kjeldahl's method, and sulphur with the nephelometric method with $BaCl_2$. All chemical analyses of both plants and soil were made in the Estonian State Centre of Agricultural Chemistry. Biomass determination on separated organs of five plants of each version of treatment was performed, their fresh mass was weighed, and their dry mass was established after they had been kept at 105 °C for 60 h. The correlation matrix of Pearson r and the significance of correlation p between the investigated parameters were calculated using the package STATGRAPHICS. For the detection of significance of differences between the results the slightly modified multiple comparison procedure according to Shirley (1987), which is frequently recommended in ecological investigations, was used.

RESULTS AND DISCUSSION

Nitrogen and sulphur allocation

There is a good review by Palumets (1991) showing the phytomass and nutrients partitioning differences in Norway spruce depending on environmental climatic factors. Air pollution may be one of the factors responsible for alterations in the patterns of the translocation of mineral nutrients and assimilates in plants.

The results of our experiments demonstrate that simulated acid mist of various pH levels changes the mineral elements accumulation in plants. A marked increase in sulphur and nitrogen content was observed in the plants exposed to elevated levels of sulphate and nitrate in the treatment solution. Broadly, nitrogen is distributed in *Phaseolus vulgaris* L. plants as follows: leaves>roots>stems (Table 2). Contrary to the nitrogen distribution, the highest content of sulphur accumulates mainly in roots, and sulphur allocation in bean plants is as follows: roots>stems>leaves (Table 2).

Treatment with solutions of pH 2.3 and 3.0 brings about essential changes of nitrogen in all organs and sulphur content in plant stems and roots as compared to the control plants. Differences between control and acid mist treated variants are given in Table 2. Correlation between the treatment pH and nitrogen and sulphur content in different organs was significant in all organs of bean plants (Table 3). Soil analyses indicated that compared with the initial point, there was a certain loss of nitrogen and sulphur content in the growth substrate during the biomass formation and development of plants in the variants treated with pH 3.0, 4.5, and 5.6 solutions, but no essential changes were detected in case of plant treatment with acid mist of pH 2.3.

Supported by the data in literature and negligible loss of nitrogen and sulphur from nutrient substrate in the last case we assumed that a certain amount of these elements may be accumulated into plants through leaves and used for the growth of plants. Although opinions about the cuticular transport properties of water, organic solutes, and ions differ there is much information on electrolytic transport across cuticles and ion exchange on their surface, which play an important role in the uptake of fertilizers by plant leaves (Becker et al., 1986; Chamel, 1986; Rinallo et al., 1986; Kisser-Briesack et al., 1987; Riederen, 1989). Evans et al. (1981, 1986) found increasing anion diffusion through cuticles of different plant species with decreasing rainwater pH. Also the rate of $^{35}SO_4^{2-}$ incorporation into bean leaves was found to increase with the decreasing pH of rainwater (Evans et al., 1981, 1985). Under controlled conditions in growth chambers Rinallo et al. (1986) demonstrated that spraying with pH 3.5 solutions of H_2SO_4 and HNO_3 resulted in an increase of both SO_4^{2-} and NO_3^- anions in young needles of *Picea abies*.

Table 2

Nitrogen and sulphur concentrations in different organs of *Phaseolus vulgaris* L. at the end of the experiment

Organs	Treatment pH	Dry matter, %	N	S	N/S
			$mg \cdot g^{-1}$ dry mass		
Leaves	5.6	9.0	26.9	1.45	18.6
	4.5	9.5	30.5 $p=0.03$	1.50	20.3
	3.0	9.4	31.9 $p=0.03$	1.60	19.9
	2.3	10.3 $p=0.01$	32.7 $p=0.03$	2.30 $p=0.03$	14.2 $p=0.01$
Stems	5.6	10.9	10.9	1.83	5.96
	4.5	11.0	13.8 $p=0.02$	2.50	5.52 $p=0.01$
	3.0	11.2	14.8 $p=0.02$	2.75 $p=0.01$	5.40 $p=0.01$
	2.3	11.7 $p=0.02$	15.4 $p=0.05$	2.85 $p=0.01$	5.40 $p=0.01$
Roots	5.6	45.8	18.2	3.90	4.67
	4.5	48.7 $p=0.02$	20.2 $p=0.03$	4.20 $p=0.05$	4.81
	3.0	59.0 $p=0.01$	21.8 $p=0.04$	4.40 $p=0.05$	4.95
	2.3	44.7 $p=0.05$	24.1 $p=0.02$	5.75 $p=0.01$	4.19 $p=0.04$

Statistical data analysis was performed within groups of plant organs (leaves, stems, roots) separately. Only significant difference of treatment from control (at level 0.05) calculated by slightly modified multiple comparison procedure according to Shirley (1987) is shown with maximum probable significance (p) level under the content value.

Table 3

Correlation coefficients (r) and their significance (p) between simulated acid mist pH and the content of mineral nutrients in different *Phaseolus vulgaris* L. organs (number of elements in correlation vector 20)

Organs of plants	N	S
Leaves	$r=-0.95$ $p<0.05$	$r=-0.80$ $p<0.05$
Stems	$r=-0.95$ $p<0.05$	$r=-0.94$ $p<0.005$
Roots	$r=-0.98$ $p<0.001$	$r=-0.85$ $p<0.05$

Consequently, the intensive rise of sulphur and nitrogen content in plant organs may be caused mainly by the impact of precipitation on plant leaves (Fig. 1). Correlation analysis of the treatment pH and sulphur concentration in plant indicated significant correlation in all organs (Table 3).

It is generally accepted that in higher plants the first step of sulphur assimilation is the activation of the sulphate ion by ATP. Further on the activated sulphate ions will be reduced to reactive -SH group. The utilization of elevated amounts of sulphur and biosynthesis of amino acids in plant tissues depend on requisite amounts of nitrogen. Within a plant cell NO_3^- ions can be reduced first to nitrite and then to ammonia. Both -SH and NH_4^+ ions will be incorporated into amino acids and finally into proteins. The adequacy of sulphur supplies to plants may be assessed from the N/S ratio. As protein synthesis requires a relatively constant ratio of N to S in optimal growth conditions of plants (Rowland et al., 1987; Kaupenjohann et al., 1989), the determination of the ratio is required if the influence of air pollution complexes containing elevated levels of SO_4^{2-} and NO_3^- ions is to be interpreted (Сергейчик, 1984; Mandre & Tuulmets, 1993).

In our experiments the N/S ratio (Table 2) tended to diminish slightly in acid-treated plant organs. This was most evident in the cases the plants were affected at pH 2.3. The change in the N/S ratio varied between plant organs. At the end of the experiment the N/S ratio in leaves was by 24% and in roots and stems by about 10% lower than in the control plants (Fig. 1). The allocation pattern of the N/S ratio between control plants' leaves, stems, and roots was 1:0.3:0.2, in acid-treated plants at pH 2.3 respectively 1:0.4:0.3.

The alterations in the N/S ratio in acid-treated plants alluded to changes in functional and structural state of plants, essentially in plant protein synthesis and in reciprocal regulation of the nitrogen and sulphur assimilation pathways (Pulmery & Moore, 1965; Rowland et al., 1987; Kaupenjohann et al., 1989; Сергейчик, 1984). The effect of acid precipitation on the regulatory interaction between assimilatory sulphate and nitrate in plants, an area about which virtually very little is known, deserves some attention in the future.

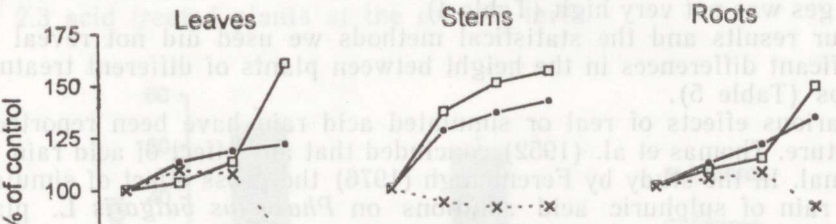

Fig. 1. Allocation of nitrogen and sulphur in the organs of *Phaseolus vulgaris* L. treated with acid mist of different pH values.

Foliar injuries and biomass allocation

Exposure to acid mist results in disbalance of the mineral composition and several functional deviations in the plant organism. These may cause changes in the biomass formation and growth of plants as secondary symptoms of impact.

Visible foliar damages of plants were first observed after 4 days since the beginning of the acid mist application at pH 2.3, and after 10 and 16 days respectively when the leaves were treated with acid mist of pH values of 3 and 4.5 (Fig. 2). Mature leaves were predominantly more susceptible to damage by acid mist than younger ones. During the whole experiment there were no visible injuries on the young trifoliate leaves.

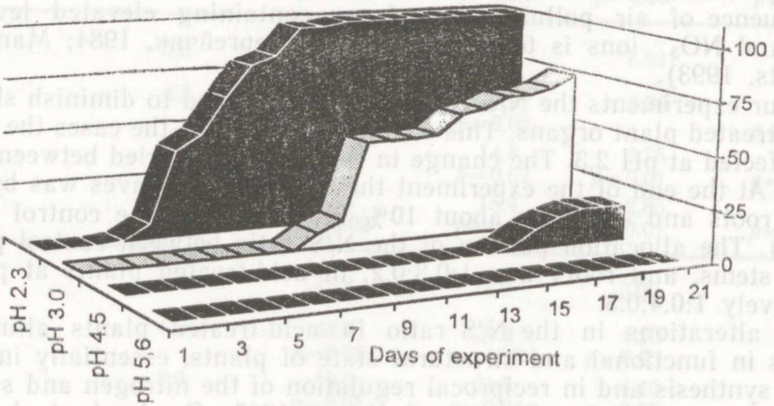

Fig. 2. Percentage of *Phaseolus vulgaris* L. plants with necrotic lesions after treatment with acid mist of different pH values (n=43).

A comparison of the necrotic leaf area and the proportion of injured leaves at different pH treatments gives some evidence that the level of damages was not very high (Table 4).

Our results and the statistical methods we used did not reveal any significant differences in the height between plants of different treatment groups (Table 5).

Various effects of real or simulated acid rain have been reported in literature. Thomas et al. (1952) concluded that any effect of acid rain was minimal. In the study by Ferenbaugh (1976) the gross effect of simulated acid rain of sulphuric acid solutions on *Phaseolus vulgaris* L. plants brought about stunted growth and interference of normal leaf development. Neufeld et al. (1985) reported that in *Platanus occidentalis* L. the height growth was significantly reduced with artificial acid rain of pH 2.0 consisting of SO_4^{2-}, NO_3^-, and Cl^-, and was stimulated with acid rain of pH 3 and 4. Insignificant reduction in height growth and a positive effect on biomass were noted by Neufeld et al. (1985) for *Robinia pseudoacacia* L. and *Liquidambar styraciflua* L. even at pH 2. This suggests that the degree of damage suffered by plants exposed to acid rain varies widely among species, depending on the age of plants, the concentration and chemical character of solutions, and the experimental conditions in modelled or natural experiments.

Table 4

Percentage of visibly injured leaves and leaf areas at the end of a 21-day experiment

Treatment pH	% of leaves injured	% of necrotic lesion areas on injured leaves
5.6	0	0
4.5	1—2	2—4
3.0	5—8	4—6
2.3	30—40	5—10

Table 5

Fresh and dry masses of different organs of *Phaseolus vulgaris* L. and average height growth of plants at the end of the experiment

Treatment pH	Height of plant, cm	Fresh mass, $g \cdot plant^{-1}$				Dry mass, $g \cdot plant^{-1}$			
		Total plant	Leaves	Stem	Roots	Total plant	Leaves	Stem	Roots
5.6	42.1	8.16	5.12	2.57	0.48	0.96	0.46	0.28	0.22
4.5	41.2	8.36	5.24	2.73	0.39 $p=0.04$	1.00	0.50	0.30	0.19
3.0	41.9	9.50 $p=0.05$	5.97 $p=0.04$	3.03	0.39 $p=0.04$	1.16	0.56 $p=0.03$	0.34	0.23
2.3	41.4	8.96	5.75	2.83	0.38 $p=0.04$	1.09	0.59 $p=0.05$	0.33	0.17 $p=0.02$

See notes to Table 2.

The relatively low doses of acid mist ($50\ ml \cdot m^{-2} \cdot d^{-1}$) applied in our experiments showed a positive effect on the leaves and stems biomass of plants. It was observed in all cases of acid mist treatment, which may be explained as a fertilizing effect of nitrogen due to the presence of NO_3^- in the simulated acid mist. However, the roots mass of *Phaseolus vulgaris* L. was inhibited, especially at the impact of acid mist of pH 2.3 (Table 5, Fig. 3). Differences were recorded between root biomass in the control and pH 2.3 acid treated plants at the $p<0.05$ level.

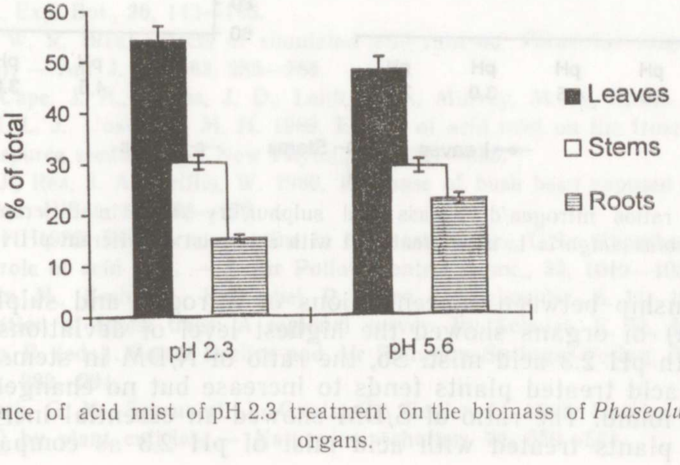

Fig. 3. Influence of acid mist of pH 2.3 treatment on the biomass of *Phaseolus vulgaris* L. organs.

Reduction in root growth in response to HNO_3 and H_2SO_4 mixture may have a secondary effect on the whole plant. Root growth reduction probably results from the reduced translocation of photosynthates to the roots in acid treated plants (Rowland et al., 1987; Mandre & Klõšeiko, 1994), which leads to a disbalance of the biomass ratio of the aboveground organs and roots (Fig. 4). The trend toward higher shoot/root ratios, caused by reduced root biomass, might predispose acid rain stressed plants to additional stresses such as drought (Johnson, 1983), infection (Ayres, 1991), and frost (Fowler et al., 1989). Earlier published results of Levin et al. (1989), who demonstrated the shoot/root increase in response to the increase of the plant nitrogen concentration, are supported by our data.

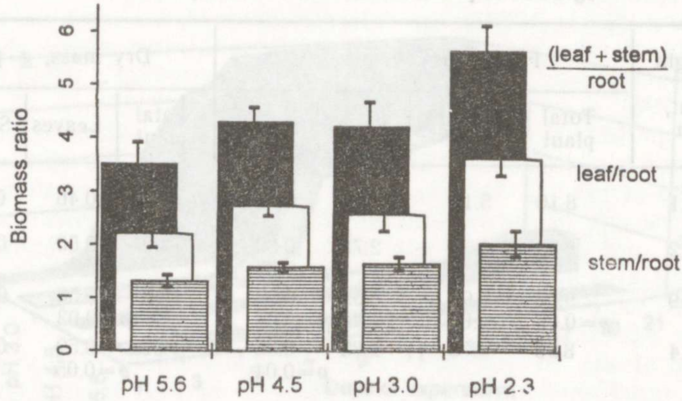

Fig. 4. The biomass ratios after treatment at different pH values. Vertical bars denote ±SEM ($n=5$).

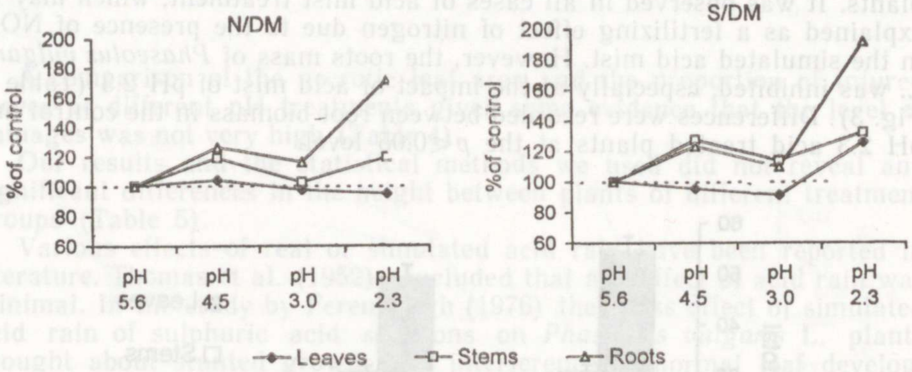

Fig. 5. The ratios nitrogen/dry mass and sulphur/dry mass in different organs of *Phaseolus vulgaris* L. after treatment with acid mist of different pH values.

Relationship between concentrations of nitrogen and sulphur to dry mass (DM) of organs showed the highest level of deviations in plants treated with pH 2.3 acid mist. So, the ratio of N/DM in stems and roots in pH 2.3 acid treated plants tends to increase but no changes in leaves have been found. The ratio of S/DM showed an essential increase in all organs of plants treated with acid mist of pH 2.3 as compared to the control plants (Fig. 5). The ratio N/DM in *Phaseolus vulgaris* L. leaves, stems, and roots is 1:0.7:1.4 in control plants and 1:0.8:2 in plants treated with acid mist at pH 2.3. The ratio of S/DM in different organs of the control plants was respectively 1:2.0:5.5 and of pH 2.3 acid treated plants 1:2.2:8.7.

The information presented in this paper indicates that there may occur changes in the allocation of mineral nutrients and biomass in plants under the influence of acid mists of rather small quantities. It was shown that to a certain degree plants are able to retain their basic allocation pattern in response processes to the impact of acid mists (pH 3 and 4.5). Our studies revealed essential changes in the allocation of nitrogen and sulphur and serious changes in the ratios N/S, N/DM, and S/DM in different organs mainly under the effect of acid mist at pH 2.3.

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