Effect of Foliar Treatment with Simulated Acid Mists on Biomass Formation and Nutrient Composition of Plants

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Z. Naturforsch. **50 c,** 521–526 (1995); received March 1/May 2, 1995

Acid Mist, *Phaseolus vulgaris* L., Mineral Nutrients, Leaf Injury, Biomass

Plants of *Phaseolus vulgaris* L. were exposed to mists 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. At the end of 21-days treatment the growth of the plants in height was not reduced; however, significant changes were detected in the mineral nutrient content and partitioning in plant organs. Treatments at pH 2.3 led to greatly decreased Ca and K content and increased S, N and P content in the leaves. In the roots the most significant increases occurred in the levels of P, S, N, K, Ca, and Mg, in the stems in the levels of N, S and P.

Introduction

The biological effects of ambient and simulated acid rain have been discussed in several excellent articles (Likens and Johnson, 1972; Almer *et al.,* 1974; Wood and Bormann, 1974; Paparozzi and Tukey, 1983; Proctor, 1983; Nygren and Hari, 1992). It was demonstrated that visible and histomorphological symptoms of foliar injury appear usually under the influence of acid rains with pH lower than 3, and this in case of realistic intensities of raining (Evans *et al.,* 1977; Evans and Curry, 1979; Adams *et al.,* 1984). Acid precipitation may cause serious physiological-biochemical changes in plants that, in turn, cause changes in growth and bioproduction. Effects of simulated acid rain on nutrient penetration, leaching and cell permeability of leaves have been studied by Evans *et al.* (1981) and Neufeld *et al.* (1985). There is information on the reduction of photosynthesis (Sheridan and Rosenstreten, 1973; Jaakkola *et al.,* 1980; Neufeld *et al.,* 1985) and increase in the average respiration rate (Ferenbaugh, 1976), also changes in mineral nutrient composition (Hindawi *et al.,* 1980), etc. have been reported. However, the mechanisms responsible for the growth alterations due to acid rain are not yet completely understood.

One of the major effects of acid rain on plants may be the altered pattern of translocation of photosynthates and mineral nutrients. This, in turn, influences the biomass formation and functional balance in plants.

The purpose of the present study was to investigate the impact of annual acid precipitation on the content and partitioning of mineral nutrients and the biomass in *Phaseolus vulgaris* L. and to detect changes in its different organs during prolonged acidic exposure.

Materials and Methods

Seeds of *Phaseolus vulgaris* L. cv. Valja were germinated in plastic pots filled with fertilized peat in a greenhouse (2 plants per pot). Then the plants were kept at 22±2 °C at daytime, at an average light intensity of 250–400 $\mu mol \times m^{-2} \times sec^{-1}$ and relative humidity of approximately 70–80%. At the beginning of the experiment the plants with well-expanded primary leaves with the central leaflet of the first trifoliate leaf were selected and distributed equally between growth chambers. The density of young plants was 43 per m^2 in each growth chamber. The plants were treated using the spraying system Englo with pH values of 2.3, 3.0, 4.5 of solutions containing H_2SO_4 and HNO_3. Control plants were sprayed with distilled water with pH 5.6. The pH of the treatment solutions was adjusted with the gradual dilution of 1.5:1 $mol \times l^{-1}$ H_2SO_4:HNO_3 stock solution in distilled water. Final acidity for each treatment was checked with laboratory pH meter. Plants were subjected to average monthly simulated acid mists

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Table I. Concentration of nutrient elements assimilable by plants in the growth substrate in the beginning and at the end of a 21-day experiment.

Treatment pH	pH*	Total N%	P_2O_5	K_2O	Ca	Mg	Cu	Zn	Fe	Mn	S
			mg×100g^{-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

* Measured in 1 N KCl.

of 2.5 mm at a frequency one treatment per day (50 ml). The duration of the experiment was 21 days. Data on the basic nutrient substrate used are presented in Table I.

Supported by the data in literature we have assumed that certain degree of nitrogen and sulphur may accumulate with acid mist through the leaves (Evans *et al.*, 1981). Depending on the concentrations of SO_4^{2-} and NO_3^- in the treatment solutions and treatment frequency the total fall-out of S and N with acid mist was calculated being respectively 88.7 and 25.9 mg×m^{-2} at pH 2.3 treatment, 12.1 and 3.5 mg×m^{-2} at pH 3.0, and 0.8 and 0.2 mg×m^{-2} at pH 4.5.

During the experiment the growth height of plants was measured and the appearance of 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 on them a transparent grid. The percent 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, Tingey and Hogsett (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 were separated for biochemical analyses and their biomasses were determined (n=5). To analyse the main mineral elements of plants (n= 10), an atom absorption analyser AAS-1N was used, nitrogen was analysed using Kjeldahl's method, phosphorus with the molybdenum blue method, sulphur with nephelometric method with $BaCl_2$. All the chemical analyses in plants and soil were carried out in the Estonian State Centre of Agricultural Chemistry. The correlation matrix of Pearson r and significance of correlation or differences P between the investigated parameters were calculated using the package STATGRAPHICS and MS EXCEL 4.0.

Results and Discussion

Foliar injury and biomass allocation

Visible foliar damages of plants were first observed in 4 days since the beginning of acid mist application at pH 2.3, after 10 and 16 days respectively when the leaves were treated with acid mist of pH values 3 and 4.5 (Fig. 1). Predominantly mature leaves were more susceptible to damage by acid mist. There were no visible injuries on the young trifoliate leaves during the whole experiment.

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. Amounts of leaves injured by acid mist treatment of pH values 4.5, 3.0 and 2.3 were respectively 1–2, 5–8 and 30–40%, but the necrotic lesion areas on injured leaves respectively 2–4, 4–6 and 5–10% at the end of a 21-day experiment.

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

Various effects of real or simulated acid rain have been reported in literature. Thomas *et al.*

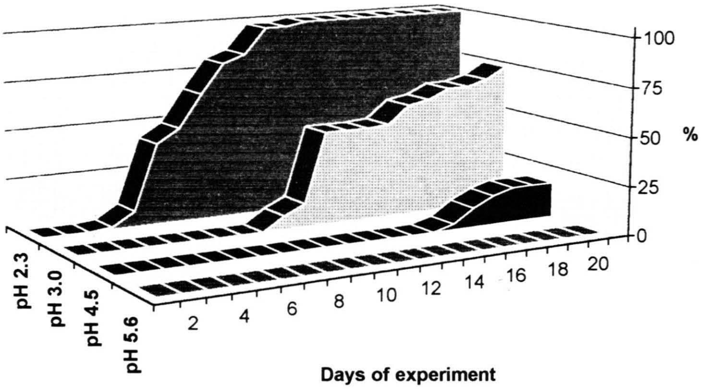

Fig. 1. Percentage of *Phaseolus vulgaris* L. plants with necrotic lesions after treatment with acid mist of different pH values (n=43).

Table II. Height and biomass partitioning of *Phaseolus vulgaris* L. plants at the conclusion of the impact of acid rain of various pH values (21-day-old plant).

Treatment pH	Height of plants [cm]	Fresh weight [g]				Dry weight [g]			
		Total plant	Leaf	Stem	Root	Total plant	Leaf	Stem	Root
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*	1.00	0.50	0.30	0.19
3.0	41.9	9.50*	5.9*	3.03	0.39*	1.16	0.56*	0.37	0.23
2.3	41.4	8.96	5.75	2.83	0.38*	1.09	0.59*	0.33	0.17*

* Level of differences from control <0.05.

(1952) concluded that any effect of acid rain was minimal. In the study by Ferenbaugh (1976) the gross effect of acid rain of sulphuric acid solutions on *Phaseolus vulgaris* L. plants included stunted growth and interference of normal leaf development. Neufeld *et al.* (1985) reported that in *Platanus occidentalis* L. height growth was significantly reduced with acid rain of pH 2.0 consisting of SO_4^{2-}:NO_3^-:Cl^-, and was stimulated with acid rain of pH 3 and 4. In significant reduction in height growth and of positive effect on biomass were noted for *Robinia pseudo-acacia* L. and *Liquidambar styraciflua* L. even at pH 2. This means that the degree of damages suffered by plants exposed to acid rain varies widely among species (Evans and Curry, 1979; Haines *et al.*, 1980), depending on the age of plants, the concentration and chemical character of solutions and the experimental conditions in modelled or natural experiments.

The relatively low doses of acid mist used in our experiments, did not allow us to detect significant changes in the biomass formation either. Only a slight positive effect on the biomass formation of plant leaves was observed, which may be explained as a fertilizing effect of nitrogen through the leaves (HNO_3 was a component of the simulated acid mist). But the roots of *Phaseolus vulgaris* L. are rather susceptible to the impact of acid mist of pH 2.3 (Table II). Some differences were recorded between root biomasses of the control and pH 2.3 acid-treated plants ($P<0.05$), which resulted in a disbalance of the ratio of the above-ground organs and roots. The trend toward higher shoot/root ratios, coupled with 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).

Partition of nutrients in plant

One of the major effects of acid rain on plants may be altering the patterns of the translocation of mineral compounds due to damages in the root system and a disbalance between the above-ground and root growth intensities.

The experimental results demonstrate that simulated acid mist of various pH levels changes essentially the mineral composition and elements partitioning in leaves, stem and roots. A marked increase in the plant sulphur and nitrogen content was observed in the plants exposed to elevated levels of sulphate and nitrate in the treatment solution. Generally, nitrogen is distributed in *Phaseolus vulgaris* L. control plants as follows: leaves > roots > stems (Table III). Treatment with

Table III. Mineral elements concentration in *Phaseolus vulgaris* L. at the end of the experiment, as a function of simulated acid mists. Correlation coefficients (r) and their significance (P) between the treatment pH and mineral elements content in organs (number of elements in correlation vector 20).

Organs	Treatment pH	Total conc. of mineral elements	K	Ca	Mg	N	S	P
		% of dry matter						
Leaves	5.6	13.4	3.53	2.4	1.58	2.69	0.145	0.221
	4.5	12.8	3.82	2.1	1.44	3.05	0.150	0.233
	3.0	14.6	3.57	2.0	1.65	3.19	0.160	0.243
	2.3	13.8	3.04	1.9	1.56	3.27	0.230	0.278
		–	–	$r=0.97$	–	$r=-0.95$	$r=-0.80$	$r=-0.80$
		–	–	$P<0.005$	–	$P<0.05$	$P<0.05$	$P<0.05$
Stems	5.6	9.5	3.74	1.09	1.10	1.09	0.183	0.133
	4.5	9.6	4.19	1.07	1.17	1.38	0.250	0.163
	3.0	9.9	3.82	1.05	1.22	1.48	0.275	0.171
	2.3	10.3	4.14	1.04	1.31	1.54	0.285	0.180
		$r=-0.86$	–	$r=0.99$	$r=-0.97$	$r=-0.95$	$r=-0.94$	$r=-0.94$
		$P<0.001$	–	$P<0.001$	$P<0.001$	$P<0.05$	$P<0.005$	$P<0.005$
Roots	5.6	8.2	2.92	1.18	2.88	1.82	0.390	0.136
	4.5	8.6	2.64	1.24	2.98	2.02	0.420	0.146
	3.0	8.8	3.10	1.42	3.52	2.18	0.440	0.186
	2.3	10.4	3.92	1.6	3.98	2.41	0.575	0.214
		$r=-0.93$	–	$r=-0.97$	$r=-0.96$	$r=-0.98$	$r=-0.85$	$r=-0.97$
		$P<0.001$	–	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.05$	$P<0.001$

solutions of pH 2.3 and 3.0 causes the biggest changes in plant stems as compared to the control. Correlation between the treatment pH and nitrogen content in plant organs is the most significant in roots (Table III). Soil investigation showed that, as compared to the initial point, there was a 20% loss of nitrogen content in the growth substrate during the experiment (Table I). In these experiments where plants were treated with acid rain of pH 2.3, no essential decrease of N content in the growth substrate was detected (Table I). This indicates that the nitrogen accumulated into plant through the leaves due to acid rain treatment is transported and used up in plant metabolism. Sulphur accumulates mainly in roots and its distribution in control plants is as follows: roots > stems > leaves (Table III). However, in the case of simulated acid mist impact the content of sulphur rose analogically to that of nitrogen and only 9% of sulphur was used from the growth substrate, while in the control 31% was used.

Consequently, the intensive rise of sulphur content in plant organs is caused mainly by the impact of polluting precipitation on plant leaves. Correlation analysis of the acid mist pH and sulphur concentration in plants indicated significant correlation in stems, while in leaves and roots it was at a level of 0.05 (Table III).

The ratio of nitrogen and sulphur tends to diminish slightly in plant organs. This was most evident in the case the plants were treated at pH 2.3. At the end of the experiment the N/S ratio in leaves was by 30% and in roots and stems by about 10% lower than in the control (Fig. 2, Table IV).

Phosphorus concentration in plants treated with acid mist of pH 2.3 and 3.0 increased (Table III), especially in roots. In the control plants the phosphorus partitioning between leaves, stems and roots showed the following ratio: 1.0:0.6:0.6. In the plants treated at pH 2.3 it the ratio was 1.0:0.8:0.7 due to redistribution. Phosphorus uptake by plants from the soil intensified in acid-treated plants approximately twice compared with the control (Table I). The N/P ratio in leaves and stems showed no essential changes, but in roots it decreased by approximately 14% compared with the control (Table IV, Fig. 2). Phosphorus concentration in plants showed a good correlation with the treatment pH (Table IV).

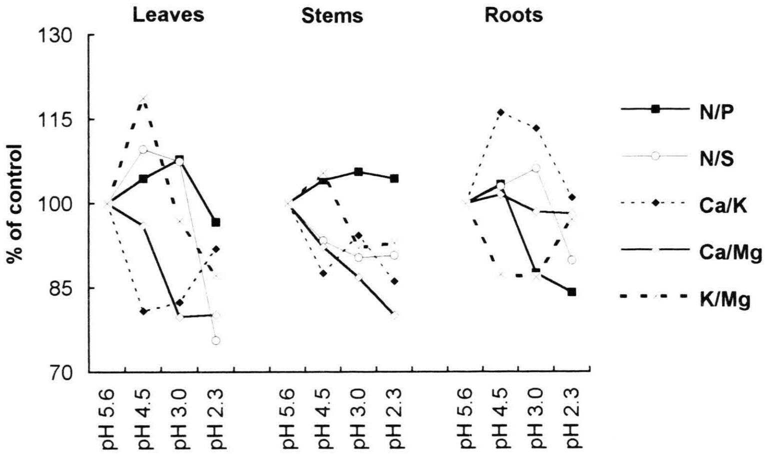

Fig. 2. Changes in nutrients ratios of *Phaseolus vulgaris* L. organs after treatment at different pH values.

Acid treatment caused significant losses in the foliar and stem calcium concentration. In roots, on the contrary, the calcium concentration increased. Potassium and magnesium amounts tended to decrease in leaves and increase in roots and stems (Table III). By the end of the experiment the acid-treated plants used calcium, potassium and magnesium from the nutrient substrate similarly to the control plants. This allows us to assume that the changes in the concentration of these elements take place due to changes in their repartition in the organism. Some researches explain the decrease of the concentration of basic elements under the influence of acid rain by leaching processes. This was demonstrated by Evans *et al.* (1981) in experiments where plants were exposed to citrate-phosphate buffers of various pH levels. Calcium was preferentially leached from leaves by rainfalls of pH 2.7. The same had been suggested earlier for potassium by Rains *et al.* (1964). In our experiments losses in foliar nutrients cannot be attributed essentially to leaching, because we used an acid solution in the form of mists with small droplets. The leaves were thoroughly wetted but droplets did not run off. The failure to detect decreased concentrations of calcium, potassium and magnesium in leaves may be explained as a redistribution of absorbed ions between plant organs or as an inhibition of their uptake by leaves.

Table IV. Ratios of mineral nutrients in *Phaseolus vulgaris* L. organs at the end of the experiment, as a function of simulated acid mists pH (% of dry matter).

Organs	Treatment pH	N/S	Ca/K	Ca/Mg	K/Mg	N/P
Leaves	5.6	18.6	0.68	1.52	2.23	12.2
	4.5	20.3	0.55	1.46	2.65	13.1
	3.0	19.9	0.56	1.21	2.16	13.1
	2.3	14.2	0.62	1.25	1.95	11.8
Stems	5.6	5.96	0.29	0.99	3.40	8.2
	4.5	5.52	0.26	0.91	3.58	8.5
	3.0	5.40	0.27	0.86	3.13	8.7
	2.3	5.40	0.25	0.79	3.16	8.6
Roots	5.6	4.67	0.40	0.41	1.0	13.1
	4.5	4.81	0.47	0.41	0.86	13.8
	3.0	4.95	0.46	0.40	0.88	11.7
	2.3	4.19	0.41	0.40	0.98	11.3

Although the results of our experiments show mainly a decrease in the calcium concentration in acid-treated leaves, there nevertheless occurs also a decrease in the concentration of all main base cations accompanied by changes in their ratios. The dynamics of Ca/K and K/Mg in the leaves and roots of experimental plants showed an opposite trend, while Ca/Mg in all plant organs tended to decrease with decreasing pH (Table IV, Fig. 2). It is well known that potassium, calcium and magnesium are needed for complete regulation of the photosynthetic and/or respiratory metabolism under acid deposition conditions (Lange *et al.*, 1989; Nygren and Hari, 1992). So, a decrease in the concentration of these elements and shifts in their ratios may influence vital functions in plants.

The information presented in this paper provides a basis for investigating the partitioning of mineral nutrients and biomass in plants under the influence of acid mists of rather small quantities at different pH levels. It was explained that to a certain degree plants are able to modify their basic partitioning pattern in response to the acidification of environment. Our studies revealed serious changes in the normal partitioning of calcium, magnesium, nitrogen, phosphorus and sulphur under the cumulative effect of different pH levels of H_2SO_4 and HNO_3. Alterations in the mobility and nutrient status may occur even in the absence of essential visible leaf injuries. The observed changes in leaves are probably mediated through the disturbed functioning of the roots.

Acknowledgement

The authors acknowledge financial support from the Estonian Science Foundation.

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