Effects of simulated acid rain on anatomy of primary leaves of *Phaseolus vulgaris*

D. STOYANOVA

Department of Botany, Faculty of Biology, Sofia University 'St. Kliment Ohridski', 8 Dragan Tzankov Str., BG-1421 Sofia, Bulgaria

Abstract

Ten-days-old bean plants (*Phaseolus vulgaris* L., cv. Cheren Starozagorski) were treated with simulated acid rain (pH 2.4, 2.2, 2.0 and 1.8). Anatomical changes in the primary leaves were studied 3, 48 and 168 h after a single treatment. This treatment induced: *1*) change in the shape of palisade cells, contraction of their contact surfaces and expansion of spongy cells (pH 1.8, 3 h after treatment); *2*) reduction of symplast connections among palisade cells and of apoplast in the spongy mesophyll (pH 1.8, 48 h after treatment); *3*) destruction of adaxial epidermis and portions of palisade mesophyll, plasmolysis of spongy cells (pH 1.8, 168 h after treatment); *4*) full destruction of mesophyll (pH 2.4, 2.2, 2.0 and 1.8, 168 h after treatment). The structure of abaxial epidermis was more stable than that of the adaxial one. With respect to anatomical parameters the studied species could be considered as comparatively resistant to acid rain.

Additional key words: abaxial and adaxial epidermes, bean, palisade and spongy parenchyma

Introduction

Acid rains as products of anthropogenic pollution consist a major problem for the countries in Europe and North America. Studies have been concentrated on grass (Bell *et al.* 1979, Swiecki *et al.* 1982, Adams *et al.* 1984) and tree species (Wood and Bormann 1974, Lee *et al.* 1993). One of the best studied group of plants in this respect are the coniferous trees. These studies concentrated on different structural and functional parameters (Bäck and Huttunen 1992, Bäck *et al.* 1993, Takemoto and Bytherowicz 1993) including epicuticular wax (Lütz *et al.* 1990, Turunen and Huttunen 1990, 1991, Turunen *et al.* 1996a, Turunen *et al.* 1996b). A significant attention was also devoted to *Phaseolus vulgaris* L. (Ferenbaugh 1976, Evans *et al.* 1977, Hindawi *et al.* 1980, Evans *et al.* 1981, Evans *et al.* 1985).

Received 24 June 1997, *accepted* 20 November 1997.
Acknowledgements: This research was supported by the National Found for Scientific Investigations (Grant MU-BAV-7) Ministry of Education, Science and Technology, Bulgaria.
Fax: (+359) 2 656641

We also used *Phaseolus vulgaris* L. plants to perform a model experiment on dosage treatment with simulated acid rain. Anatomical features were considered as sensitive indicator of damage occurring before visible damage (necroses) occur. The study was expected to shed light on the threshold levels of toxicity of simulated acid rain as well as on the possible reaction of plants to a real acid rain in natural conditions. The present anatomical study is a part of a complex structural and functional study on the influence of simulated acid rain on bean plants.

Materials and methods

Seeds of bean plants (*Phaseolus vulgaris* L., cv. Cheren Starozagorski) were germinated in moist sand. Seedlings were grown in containers (capacity 1500 cm^3) filled firstly with distilled water and after 2 d with Knop nutrient solution placed in a climatic chamber (12-h photoperiod, temperature 25 °C, air humidity 70 %, photon flux density 120 μmol m^{-2} s^{-1}) Ten-days-old plants were treated (sprayed) once with simulated acid rain (cocktail according to Seufert *et al.*, 1990) of pH 2.4, 2.2, 2.0 and 1.8, respectively. Plants of the same age but treated with solution of pH 5.6 were used as a control. The anatomy of the primary leaves was studied after 3, 48 and 168 h.

Segments from the middle parts of the primary leaf were fixed in 3 % glutaraldehyde and used for light microscopy studies. The thickness of the lamina, the mesophyll, the palisade and spongy parenchyma were measured. Leaves were cut by hand. Ten morphometric measurements were repeated in triplicate and microphotographs were taken using *Amplival 4* microscope (*Carl Zeiss*, Jena, Germany).

The comparison of quantitative histological data of 17-d-old and 10-d-old control plants clearly revealed that the primary leaves of 10-d-old control plants were entirely differentiated. These results encouraged us using 10-d-old plants for experiments.

Results and discussion

The primary leaf of beans is bifacial (dorsiventral), amphistomatic (Fig. 1). Stomatal type is anomocytic. The mesophyll is differentiated into a one layer palisade parenchyma and 4 - 5 layers of spongy parenchyma. The thickness of lamina and mesophyll in primary leaf of 10-d-old plants were 183.14 ± 12.70 μm and 144.53 ± 13.04 μm, respectively (Table 1). The mesophyll comprised 79 % of the total lamina thickness. Spongy parenchyma occupied bigger share of the lamina possessing mean thickness 95.57 ± 12.03 μm. The palisade factor (the ratio of thickness of palisade parenchyma and thickness of mesophyll in %) was 36.2 % (Table 1).

Leaves were treated with simulated acid rain of different pH: 2.4, 2.2, 2.0 and 1.8. The morphometric analysis of the anatomical parameters was carried out in zones of the leaf lamina which were not visibly damaged. The plants subjected to simulated acid rain of pH 1.8 possessed strongly decreased pressure potential of leaves 3 h after treatment. Nevertheless, the average thickness of leaf lamina was 9 % higher than that of the control plants (199.28 ± 17.67 μm) (Table 1). Palisade and spongy tissues in treated variant comprised 28 and 50 % from the total thickness of the lamina, respectively, in comparison with 28 and 52 % in the control variant. This indicated that no significant quantitative changes took place in the mesophyll. Bäck *et al.* (1994) also established no statistically significant differences in the leaves of coniferous plants subjected to acid treatment. Evidently, morphometric data could not

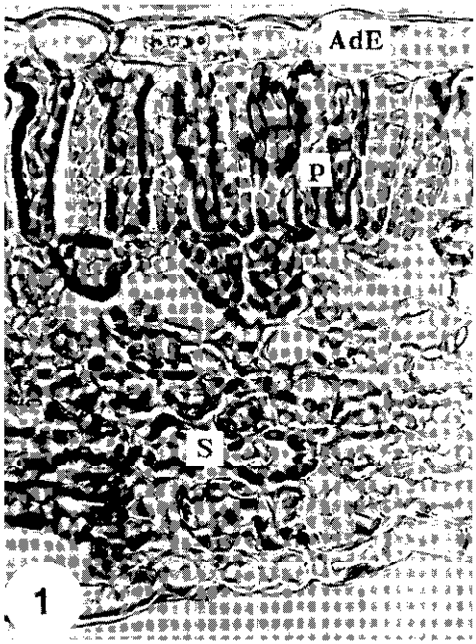
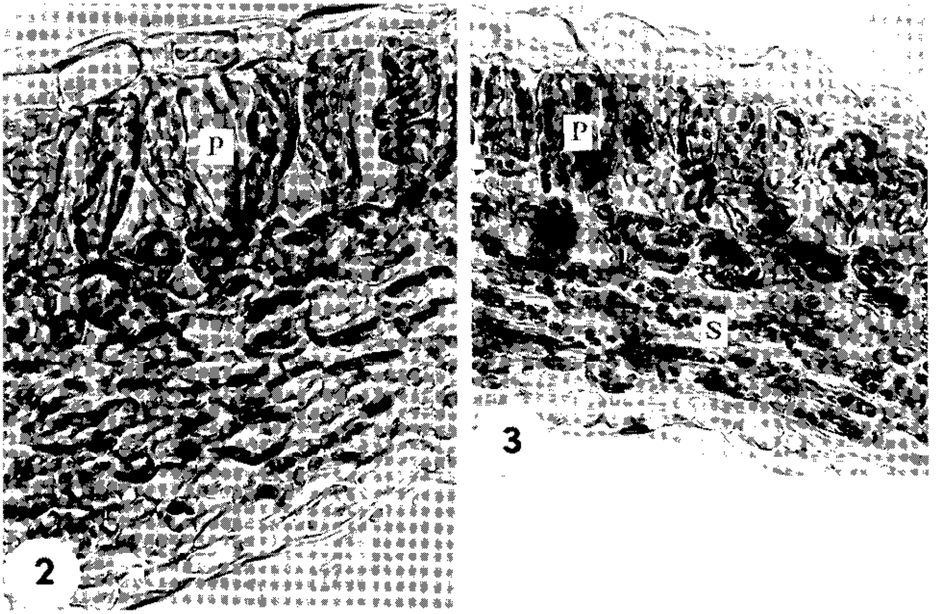


Fig. 1. Anatomical structure of primary leaf in 10-d-old control bean plants (AbE - abaxial epidermis, AdE - adaxial epidermis, P - palisade parenchyma, S - spongy parenchyma) [× 160].

Table 1. The thickness [μm] of leaf lamina and its tissues, and palisade factor [%] in bean plants treated with simulated acid rain of different pH and measured 3, 48 or 168 h after treatment.

		Lamina	Mesophyll	Palisade parenchyma	Spongy parenchyma	Palisade factor
control	10-d-old	183.14±12.70	144.53±13.04	52.28± 3.98	95.57±12.03	36.2
	17-d-old	187.17±18.77	149.67±35.52	53.35± 3.75	97.71±33.55	35.6
pH 1.8	3 h	199.28±17.67	148.82± 9.17	55.07± 5.07	101.25±13.85	37.0
	48 h	180.28±12.79	144.21±16.13	53.35± 7.36	90.85±14.12	36.9
	168 h	187.14±23.59	134.89±28.24	49.07±14.79	89.35±20.62	36.4
pH 2.0	168 h	195.42±19.33	146.03±25.47	55.50± 8.00	93.75±24.17	38.0
pH 2.2	168 h	154.57±16.04	112.60±10.80	41.46± 4.30	70.17±12.18	36.8
pH 2.4	168 h	182.85±13.80	149.25±24.12	51.64± 5.38	98.57±23.09	34.6

provide sufficient information concerning the type and degree of anatomical changes in the zones without visible damages. Taking into account the conclusion of Adams *et al.* (1984) that the visual evaluation of leaf surface could lead to a significant underestimation of tissue damages, detailed microscopic observations were carried out. In the sections possessing similar quantitative anatomy with the control plants, specific changes in the palisade and spongy parenchyma were registered (Figs. 2, 3). The most frequently observed change in the palisade parenchyma were changes in cell shape to very twisted forms and space orientation within the tissue (Fig. 2). This led to reduction of their contact surfaces and increase of intracellular distances which could also be observed by surface cuts. Using histochemical methods Bäck *et al.* (1993) also established deformations in mesophyll cells of coniferous trees after treatment with pH 3.0. In the nearest vicinity of the chlorotic zones histological changes were more pronounced (Fig. 3): *1*) the leaf lamina was thinner, 2) the cells of palisade parenchyma were significantly shorter and more densely situated, and 3) the spongy cells were tangentially flattened. Similar changes in mesophyll of bean plants were observed after treatment with excess copper (Maksymiec *et al.* 1995) and therefore, they were not specific for acid rain damages.

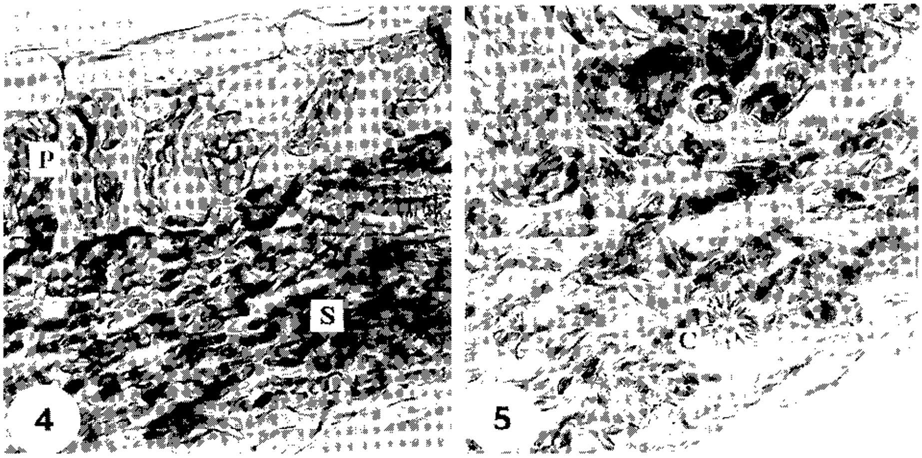


Figs. 2 and 3. Changes of the structure of primary leaf in plants treated with pH 1.8 (3 h after treatment). Fig. 2. Change in the shape of palisade cells (P) and reduction of their contact surfaces [× 160]. Fig. 3. Shortening of palisade cells (P) and tangential flattening of spongy cells (S) [× 160].

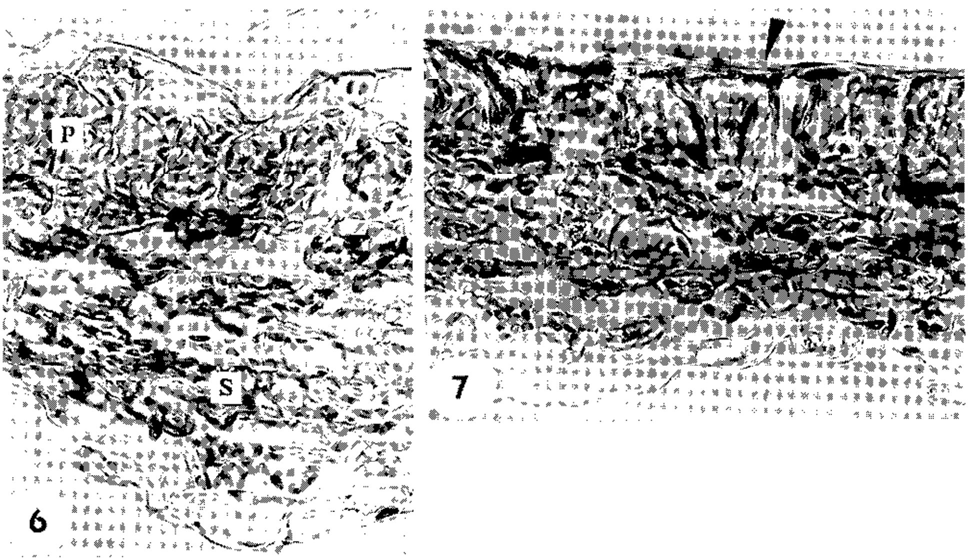
48 h after the treatment with pH 1.8 the average thickness of lamina in the zones without visible damage was also approximately the same as that of the control plant (180.28 ± 12.79 μm). The changes in the mesophyll were more pronounced but of the same nature like after 3 h (Fig. 4). The cells of the palisade tissue had non-specific irregular shape and their contact surfaces were reduced to the maximum. The spongy cells were tangentially flattened and the tissue almost lacked intracellular space.

Crystal geodes were established in the intracellular space of the spongy tissue (Fig. 5) probably residuals of cocktail components.

168 h after treatment with pH 1.8, large necrotic zones appeared on the leaf surface. Nevertheless, the average thickness of leaves in the visibly undamaged parts



Figs. 4 and 5. Changes in the structure of primary leaf in plants treated with pH 1.8 (48 h after treatment). Fig. 4. Strong change in the shape of palisade cells (P) and maximum reduction of their contact surfaces. Tangentially flattened spongy cells (S) [× 160]. Fig. 5. Crystal geodes (C) in the intracellular space of spongy parenchyma [× 160].



Figs. 6 and 7. Changes in the structure of primary leaf in plants treated with pH 1.8 (168 h after treatment). Fig. 6. Changes in the palisade (P) and spongy parenchyma(S) [× 160]. Fig. 7. Destruction of the adaxial epidermis cells (*arrow*) [× 160].

of lamina did not change significantly. The palisade (26 %) and spongy (47.7 %) tissues possessed a smaller share in it, though the palisade factor (36.4 %) did not change. The mesophyll of primary leaf was, however, built up of short closely situated palisade cells and tangentially flattened spongy cells (Fig. 6). In a great part of the leaf surface the action of acid rain caused damage to the cells of the adaxial epidermis (Fig. 7, *arrow*). Some of the palisade cells were destroyed and the integrity of the tissue was broken. The spongy cells expanded and part of them underwent plasmolysis. Similar changes of abaxial epidermis and mesophyll as a result of acid treatment were observed by Swiecki *et al.* (1982) in 12-d-old bean plants subjected to the 0.06 M hydrochloric acid and Lee *et al.* (1993) in *Quercus acutissima* seedlings treated with solutions of pH 2.5 and 4.0. The adaxial epidermis was much more resistant. These results were not in accordance with our microscopic analysis of acid treated bean plants where we established very low resistance of adaxial epidermis. The picture of pathological changes was more clear after analysis performed in plants treated simulated acid rain of higher values of pH (2.4, 2.2 and 2.0) and the same duration of treatment (168 h). We succeeded to register the diferent stages of necrotic processes taking place in the tissues of leaves treated with pH 2.0 (Figs. 8, 9 and 10).

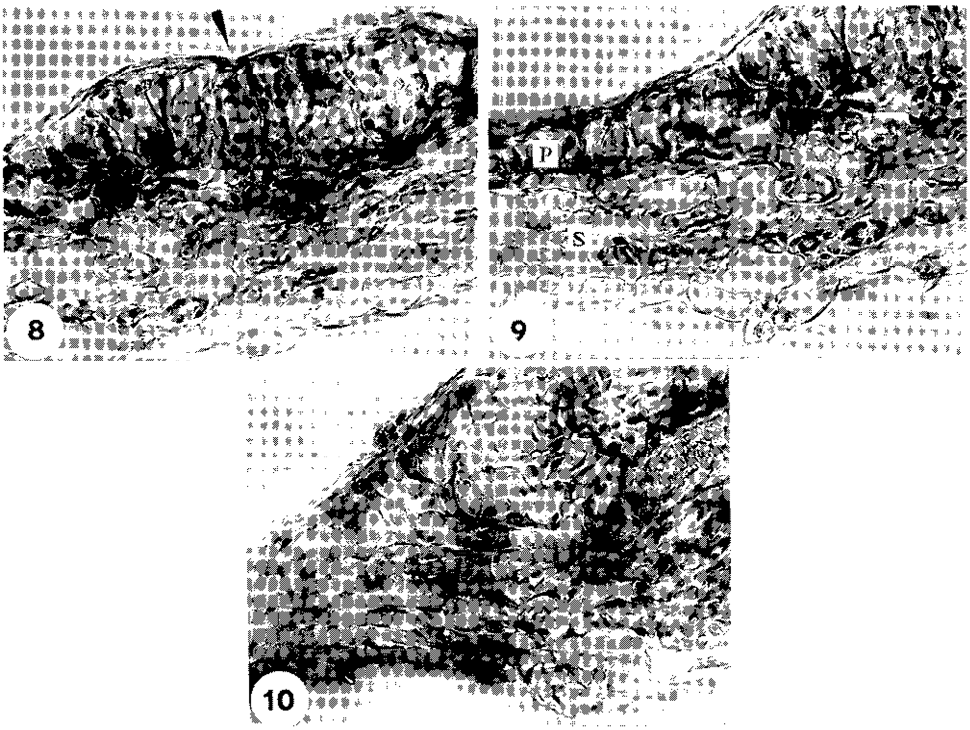


Fig. 8. Destruction of the adaxial epidermis (*arrow*) [× 160].

Fig. 9. Destruction of palisade (P) and spongy parenchyma (S) [× 160].

Fig. 10. Transition between inner destruction and outer visible necrotic changes [× 160].

In the treated plants the anatomical structure in some portions of the leaf lamina was close to the control. In the rest the gradual necrotization of tissues was registered. The damage of adaxial epidermis was considered as a primary stage of destruction (Fig. 8, *arrow*). In these sections the cells of mesophyll were deformed with atypical orientation of the tissue. Almost all spongy cells underwent plasmolysis. In these zones the lamina was thinner than that of control plants. In the zones situated closely to the necrotic spots, the destructive changes were better pronounced (Fig. 9). As a result of destruction it was difficult to draw a dividing line between palisade and spongy parenchyma, the latter not consisting already of 4 - 5 layers as usual. The thickness of these parts of lamina was even more reduced. This degree of destruction of tissues could be considered as final phase of inner damage after which total necrosis occurs (Fig. 10). This damage of mesophyll was also observed by Reinikainen and Huttunen (1989) in spruce treated with pH 4.0. The authors characterised it as total disorganisation and collapse of the mesophyll cells. When both leaf surfaces were treated equally, full destruction of adaxial epidermis and mesophyll took place, but no destroyed cells were observed in the abaxial epidermis. The explanation was probably the differences in trichomes (Evans *et al.* 1977) and stomata density on the upper and lower surface. Using SEM analysis, Adams *et al.* (1984) observed open stomata on the highly damaged zones on the upper surface of *Artemisia tilesii* leaves subjected to acid rain with pH 2.5. It was most probable that the early damage of the trichomes and the epidermis cells around stomata led to a collapse in this part of the epidermal tissue. These results support the observations of Tamm and Cowling (1977) that acid rains affect gas exchange and transpiration. Similarly, Evans *et al.* (1981) established deviations in gas exchange and high stomatal conductance of the adaxial epidermis *Phaseolus vulgaris* under pH 2.7. It seems that the destruction of adaxial epidermis could be considered as an initial stage of damage followed by changes in the palisade and later in the spongy mesophyll. Destruction of tissues in this sequence was registered also by Adams *et al.* (1984) in *Artemisia tilesii* in natural conditions (pH of rains 3.0 and 2.5). It makes us believe that from our model experiment we have obtained data close to the natural conditions. Adams *et al.* (1984) defined the range of pH 3.0 - 3.2 to 2.5 as the most toxic for acid treatment of leaves. Microscope studies indicated that there exists a large group of plants highly sensitive to acid treatment including coniferous trees which possess threshold level of pH about 4.0 (Reinikainen and Huttunen 1989, Turunen *et al.* 1995). We assume that a species like *Phaseolus vulgaris* could be considered comparatively resistant to acid treatment under natural conditions if it possessed threshold level of pH about 2.0.

References

Adams, C.M., Dengler, N.G., Hutchinson, T.C.: Acid rain effects on foliar histology of *Artemisia tilesii*. - Can. J. Bot. **62**: 463-477, 1984.

Bäck, J., Huttunen S.: Structural responses of needles of conifer seedlings to acid rain treatment. - New Phytol. **120**: 77-88, 1992.

Bäck, J., Huttunen, S., Kristen, U.: Carbohydrate distribution and cellular injuries in acid rain and cold-treated spruce needles. - Trees **8**: 75-84, 1993.

Bäck, J., Neuvonen, S., Huttunen, S.: Pine needle and fine structure after prolonged acid rain treatment in the subarctic. - Acta Univ. Oul. **A254** (V): 1-24, 1994.

Bell, J.N.B., Rutter, A.J., Relton, J.: Studies on the effects of low levels of sulphur dioxide on the growth of *Lolium perene* L. - New Phytol. **83**: 627-643, 1979.

Evans, L.S., Gmur, N.F., Da Costa, F.: Leaf surface and histological perturbations of leaves of *Phaseolus vulgaris* and *Helianthus annuus* after exposure to simulated acid rain. - Amer. J. Bot. **64**: 903-913, 1977.

Evans, L.S., Curry, T.M., Lewin, K.F.: Responses of leaves of *Phaseolus vulgaris* to simulated acid rain. New Phytol. **88**: 403-420, 1981.

Evans, L.S., Santucci, K.A., Patti, M.J.: Interactions of simulated rain solution and leaves of *Phaseolus vulgaris* L. - Environ. exp. Bot. **25**: 31-40, 1985.

Ferenbaugh, R.W.: Effects of simulated acid rain on *Phaseolus vulgaris* L. (*Fabaceae*). - Amer. J. Bot. **63**: 283-288, 1976.

Hindawi, I.J., Rea, J.A., Griffits, W.L.: Response of bush bean exposed to acid mist. - Amer. J. Bot. **67**: 168-172, 1980.

Lee, K.H., Chung, G.C., Lee, J.S.: Effects of simulated acid rain on stomatal resistance, wettability and anatomical changes in *Quercus acutissima* and *Ginkgo biloba* seedlings. - J. korean Forest. Soc. **82**: 337-346, 1993.

Lütz, C., Hinzmann, U., Gülz, P.G.: Surface structures and epicuticular wax composition of spruce needles after long-term treatment with ozone and acid mist. - Environ. Pollut. **64**: 313-322, 1990.

Maksymiec, W., Bednara, J., Baszynski, T.: Responses of runner bean plants to excess copper as a function of plant growth stages: Effects on morphology and structure of primary leaves and their chloroplast ultrastructure. - Photosynthetica **31**: 427-435, 1995.

Reinikainen, J., Huttunen, S.: The level of injury and needle ultrastructure of acid rain - irrigated pine and spruce seedlings after low temperature treatment. - New Phytol. **112**: 29-39, 1989.

Seufert, G., Hoyer, V., Wollmer, H., Arnolt, U.: The Hohenheim long-term experiment. General methods and materials. - Environ. Pollut. **68**: 205-229, 1990.

Swiecki, T.J., Endress, A.G., Taylor, O.C.: Histological effects of aqueous acids and gaseous hydrogen chloride on leean leaves. - Amer. J. Bot. **69**: 141-149, 1982.

Takemoto, B.K., Bytnerowicz, A.: Effects of acidic fog on seedlings of *Pinus ponderosa* and *Abies concolor*: foliar injury, physiological and biochemical responses. - Environ. Pollut. **79**: 235-241, 1993.

Tamm, C.O., Cowling, E.B.: Acidic precipitation and forest vegetation. - Water Air Soil Pollut. 7: 503-511, 1977.

Turunen, M., Huttunen, S.: A review of the response of epicuticular wax of conifer needles to air pollution. - J. Environ. Qual. **19**: 35-45, 1990.

Turunen, M., Huttunen, S.: Effect of simulated acid rain on epicuticular wax of Scots pine needles under northerly conditions. - Can. J. Bot. **69**: 412-419, 1991.

Turunen, M., Huttunen, S., Bäck, J., Lamppu, J.: Acid rain-induced changes in cuticles and Ca distribution in Scots pine and Norway spruce seedlings. - Can. J. Forest Res. **25**: 1313-1325, 1995.

Turunen, M., Huttunen, S., Percy, K.E., McLaughlin, C.K.: Epicuticular wax of subarctic Scots pine needles: response to ageing, sulphuric acid and heavy meals. - Acta Univ. Oul. **A277** (V): 1 28, 1996a.

Turunen, M., Huttunen, S., Percy, K.E., McLaughlin, C.K., Lamppu, J.: Structure, chemical composition and wettability of Scots pine needles epicuticular wax in a northern smelter area. - Acta Univ. Oul. **A277** (VI): 1-20, 1996b.

Wood, T., Bormann, F.H.: The effects of an artificial acid mist upon the growth of *Betula alleghaniensis* (Britt.). - Environ. Pollut. 7: 259-268, 1974.