Effects of simulated acid rain on the photosynthetic characteristics of *Phaseolus vulgaris* L.

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Abstract

A single treatment with a low pH solution of bean plants led to serious changes in the net photosynthetic rate (P_N) as well as in various parameters of photosystem 2 (PS2) activity. A considerable suppression of P_N was established already in the first hours (3 and 5) following the acid treatment (pH 2.4-1.8). The period of strong inhibition of CO_2 uptake and photochemical activity was followed by the period of recovery (24-72 h). At a single spraying, pH values exceeding 2.0 did not lead to irreversible damages of the photosynthetic apparatus. The damages resulting from treatments with pH 2.0 and 1.8 were on the threshold of irreversible ones and were the cause of faster ageing.

Additional key words: chlorophyll fluorescence; French bean; net photosynthetic rate; photochemical activity; stomatal conductance; transpiration rate; water use efficiency.

Introduction

Serious damages caused by the so-called acid rains are due to anthropogenic contaminations of the environment with sulphur and nitrogen compounds. The acid rains cause damages on the environment even in regions distant from the direct source of emission. Usually, the effect of acid rain with pH of 3.0-4.0 on the plants is weak or not observable. Neufeld *et al.* (1985) show that under greenhouse conditions only the rain of an extremely low pH causes foliar damage and growth reductions. At these acidities, in the presence or absence of visible injuries, plant development can

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Abbreviations: Chl - chlorophyll; F_0 - initial Chl fluorescence; F_v - variable Chl fluorescence; F_m - maximal Chl fluorescence; g_s - stomatal conductance; IK - induction kinetics; LHC2 - light-harvesting Chl *a/b* protein complex(es); OID phase - fast phase of prompt Chl fluorescence kinetics; P_N - net photosynthetic rate; PS - photosystem; PSET - photosynthetic electron transport; Q_A, Q_B - primary and secondary quinone acceptors of PS2; WUE - water use efficiency.

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be stimulated (Irving and Miller 1980, Lee *et al.* 1981, Shelburne *et al.* 1993), depressed (McLaughlin *et al.* 1993, Sheppard *et al.* 1993, Dixon and Kuja 1995, Muthuchelian *et al.* 1995) or not influenced (Wood and Bormann 1974, Ferenbaugh 1976). Hogan and Taylor (1995) found that the acid precipitation of pH 3.5 did not act as a primary stress. Simulated acid rain (pH 3.0-2.3) increased the Chl contents in *Pinus armandy*, decreased P_N, but did not alter the carbon allocation and biomass accumulation (Shan *et al.* 1995, 1996). In isolated chloroplasts from two tropical tree species a decrease in the PS2 whole electron transport chain activity was observed only at pH values of 3.0 and 2.0, but no significant change in PS1 activity was measured (Muthuchelian *et al.* 1995). The main action site of various phytotoxicants is the photosynthetic apparatus (Osmond 1981, Miyake *et al.* 1984, Hogan 1992).

The acid rains are composed mainly of sulphur dioxide, nitrogen oxides, ammonia, and ammonium sulphate. Entering through stomata or cuticule, SO_2 in leaves is oxidized to SO_3^{2-} and HSO_3^- which are toxic (Ziegler 1975, Peiser and Yang 1985). A considerable suppression of photosynthesis following action of SO_2 or its anionic forms was reported by Silvius *et al.* (1975), Anderson and Duggan (1977), Daniell and Sarojini (1981), Cerović *et al.* (1982), Shimazaki *et al.* (1984), Saraswathi and Madhava Rao (1995), *etc.*

The behaviour of NO_2 is analogous to that of SO_2: NO_2 in the leaves is oxidized to NO_3^- and the more toxic NO_2^-. Many plants have enzyme mechanisms for their detoxication to a determined level. NO and NO_2 in concentrations not leading to the appearance of visible damages cause also inhibition of the CO_2-fixation (Hill and Bennett 1970, Capron and Mansfield 1976, Saxe 1986). The combined action of these gases is additive, although in comparison with the NO_2 effect the NO-effect was expressed quicklier (Hill and Bennett 1970). Once the gas action has stopped, the plant photosynthetic activity is restored (Hill and Bennett 1970, Bennett and Hill 1975). White *et al.* (1974) and Carlson (1983) show that P_N functions of *Medicago sativa* and *Glycine max* are synergistically inhibited by the action of SO_2-NO_2 mixture containing low concentrations of these gases. In *Pisum sativum*, the additive effect of SO_2 and NO_2 results in photosynthesis suppression (Bull and Mansfield 1974).

Various SO_2 and NO_2 ion forms may be the source for formation of other active oxygen radicals ($^1O_2$, $OH\cdot$, H_2O_2) that are exceptionally reactive and cause the peroxidation of lipids, protein denaturation, DNA mutations, and as a consequence, changes in the plant genotype (Halliwel and Gutterige 1989). PS2, the link of photosynthetic electron transport chain reaction most vulnerable to any stress, is the target for SO_2 action (Shimazaki *et al.* 1984, Covello *et al.* 1989). SO_2 suppresses the PS2 activity and inhibits the non-cyclic photophosphorylation, whereas the cyclic photophosphorylation is not affected (Shimazaki *et al.* 1984).

The aim of our investigation was to characterize the responses of photosynthetic apparatus in bean plants to simulated acid rains, to clarify some of their toxic action mechanisms, and also to specify the pH threshold at which the damage becomes irreversible.

Materials and methods

Plants: Beans (*Phaseolus vulgaris* L. cv. Cheren Starozagorsky) were grown in 1 500 cm^3 glass vessels on Knop's nutrient solution in a climatic chamber at 23-25 °C, photon flux density (PFD) 120 μmol m^{-2} s^{-1}, and 12/12 h day/night photoperiod. Each vessel was covered with a lid which did not permit the acid rain to contaminate the nutrient solution. During the experiment, pH of the solution was checked upon in order to verify whether it changed as a result of the ion release from roots, but no such changes were registered. Ten-day-old plants were separated to variants and sprayed only once.

For simulation of acid rain the solution proposed by Seufert *et al.* (1990) was used. It contained [kg m^{-3}]: NH_4NO_3 1.3, $MgSO_4 \times 7\ H_2O$ 3.1, Na_2SO_4 2.5, $KHCO_3$ 1.3, $CaCl_2 \times 2\ H_2O$ 3.1. Its pH was adjusted to appropriate values with 1 N H_3PO_4 and 1 N H_2SO_4.

Gas exchange and Chl fluorescence measurements were taken 3, 5, 24, 48, 72, and 168 h after treatment. P_N and g_s were measured using a photosynthetic measurement system *LI-6000* (*LiCor*, USA), Chl fluorescence induction kinetics (IK) with a pulse modulated fluorimeter *PAM* (*H. Walz*, Germany). For analysis of the IK parameters, a sequence of two pulses of exciting radiation were used: the first IK was registered on 3 min dark-adapted leaves at a low actinic irradiation (60 μmol m^{-2} s^{-1} PFD, 5 s duration). The second pulse with a saturating irradiation (over 3000 μmol m^{-2} s^{-1} PFD, 2 s duration) was effected after an additional 2 min dark period. At the low irradiance the IK showed a well-pronounced fast phase designed by Papageorgiou (1975) as the OID phase (in our investigation F_i is fluorescence at the inflex point I, which correlates with the fluorescence of closed Q_B nonreducing PS2 centres). After an additional high irradiance pulse (2 s), the maximal fluorescence (F_{im}) tallied with the maximal fluorescence of all PS2 centres in the probe. Thus, the ratio $[dF_i\text{-}dF_{im}] = (F_i-F_0)/(F_{im}-F_{i0})$ was used as a criterion for the relative concentration of the Q_B nonreducing centres, and relative variable fluorescence during radiation pulse $(F_{im}-F_{i0})/F_{i0}$ as a measure of photochemical activity (Yordanov *et al.* 1997). The IK was recorded and analyzed by a computer program *FIP 4.3* (Tyystjärvi and Karunen 1990).

Statistics: All results were represented as means ± SE from at least 3 independent series of experiments (3-5 measurements each). The significant differences were determined by the Student's *t*-test.

Results

Effective pH of acid rain: In order to establish this value, solutions of three pH values were tested: pH 5.6 as a control (contained all components of simulated rain except H_2SO_4 and H_3PO_4), pH 4.6 inducing a mild stress, and pH 2.6 causing a strong (acute) stress. Our measurements of P_N and photochemical activity showed that the mixtures of pH 4.6 and 2.6 did not cause noticeable changes in the photosynthetic apparatus of bean (Table 1). These results could be explained by an increased buffer capacity of bean cells observed by Craker and Bernstein (1984) who compared the capacity of *P. vulgaris*, *Glycine max*, *Trifolium pratense*, *Triticum aestivum*, *Zea mays* and *Phleum pratense*. After 1 h incubation in solutions of pH 3.99, 2.99, and 1.99, bean leaves had the highest buffer capacity which protected plants from serious injuries by acid rain. The results of our preliminary experiments gave us reason to follow also the influence of acid rain with a pH lower than 2.6, *i.e.*, 2.4, 2.2, 2.0, and 1.8, in order to establish the pH threshold for irreversible injury of the photosynthetic apparatus.

Table 1. Influence of simulated acid rain on net photosynthetic rate (P_N) [$\mu g(CO_2)\ m^{-2}\ s^{-1}$] and photochemical activity [relative].

pH	Time after treatment [h] 24	48	72
	P_N		
5.6	497 ± 43	515 ± 34	466 ± 19
4.6	489 ± 27	521 ± 33	498 ± 1
2.6	452 ± 39	482 ± 7	492 ± 25
	Photochemical activity		
5.6	424 ± 7	388 ± 3	392 ± 5
4.6	420 ± 8	391 ± 4	399 ± 5
2.6	418 ± 11	363 ± 9	383 ± 8

Visible damages in the first hours after treatment were observed in plants sprayed with the solution of pH 1.8. After 3 h, these plants had a strongly reduced pressure potential. Chlorotic spots appeared on the majority of leaf surfaces. Five hours after the treatment, these spots extended and were seen on both upper and bottom surfaces. Single chlorotic spots were also found on the leaves of plants treated with pH of 2.0. Some singly situated necrotic points were observed on the leaves of plants sprayed with pH values of 2.2 and 2.4, 48 h after the treatment: 12 % of the leaves were damaged. Necrotic spots were observed on 40 % of the leaves treated with pH of 2.0 at the 48th h, and at the 72nd h, necrosis was noted on all leaves. The most considerable damages ensueing from the action of pH 1.8 resulted in necrotic spots on all leaves after 48 h, and after 72 h they progressed to larger necrotic sections. Withering of leaf blade edges was also observed.

Gas exchange: All the treatments inhibited P_N considerably: the largest inhibitions were observed after 5 h (Fig. 1). The inhibition increased with lowering pH: at pH of 1.8, P_N was almost completely inhibited after 5 h (Fig. 1*B*). Under some treatments the values went below the CO_2 compensation concentration. 48 and 72 h after the treatment a certain reparation of photosynthetic activity was observed (Fig. 1*D,E*). Although necrotic spots were formed on the leaves of plants treated with pH of 1.8, the remaining undamaged part functioned more intensively in order to compensate the contribution of necrotized parts. A similar feature was described by Edelman

(1933) and Ïordanov (1979) at artificial defoliation. Yet after a certain stabilization of P_N at the 72nd h, it diminished strongly at the 168th h (Fig. 1*F*).

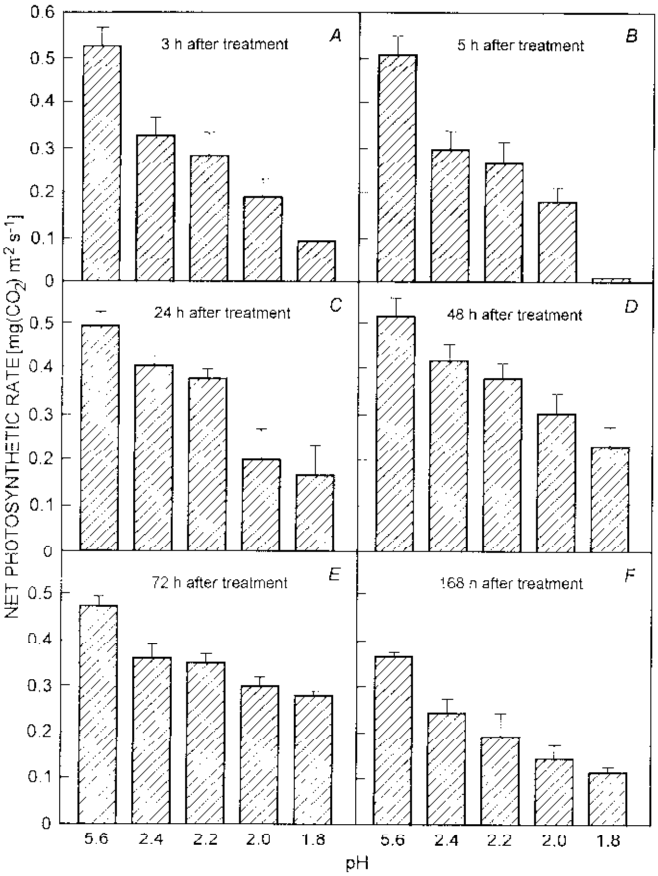

Fig. 1. Influence of simulated acid rain of different pH and duration on the net photosynthetic rate of primary bean leaves. The net photosynthetic rates were measured on attached leaves by means of *LI-6000* at 25-27 °C, 800 μmol m^{-2} s^{-1}, and 400 cm^3(CO_2) m^{-3}. The values are means ±SE at least of 3 experiments (5 measurements each). pH 5.6 means control.

Transpiration rate and stomatal conductance (g_s) were also strongly affected during the first (3 and 5) hours after spraying (Table 2). A good correlation was established between the decrease in P_N and g_s. A low pH rain (pH 2.0 and 1.8) caused significant reduction of water use efficiency (WUE) at 3 and 5 h after the treatment (Table 2). After the recovery period (168 h), WUE decreased again in the variants with pH values of 2.0 and 1.8.

Chl fluorescence: Spraying the leaves with a solution of pH 1.8 led in the first hours to a change in the shape of fluorescence IK and to a significant decrease of F_v. This was related to thylakoid membrane damages and inhibition of photosynthetic electron transport (PSET) rate. Treatment with a pH higher than 2.2 did not result in such considerable changes. The simulated acid rain treatment led to a strong reduction of photochemical activity in plants sprayed with pH 1.8 even at the 3rd and 5th hour (Fig. 2*A*,*B*) followed by a recovery that was highest at the 72nd h (Fig. 2*E*). The ratio

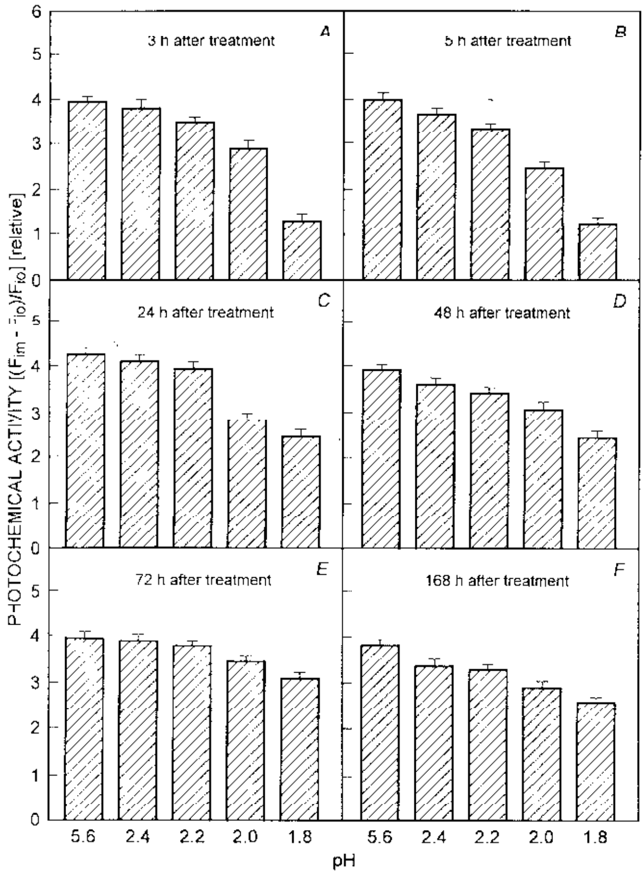

Fig. 2. The response of primary bean leaf photochemical activity, evaluated by relative variable chlorophyll fluorescence [$(F_{im} - F_{i0})/F_{i0}$] ratio, to simulated acid rain of different pH and duration. pH 5.6 means control.

$(F_i-F_0)/(F_{im}-F_{i0})$ gives information on the relative part of Q_B non-reducing centres. 3 to 5 h after treatment of plants with pH 1.8 (Fig. 3*A*,*B*) the relative part of Q_B non-reducing centres increased significantly: it was up to 3-fold higher than in the controls. The values of this parameter were fairly high until the 24th h. The relative part of Q_B non-reducing centres also increased at pH of 2.0 but to a lesser extent.

The recovery of P_N (Fig. 1*C*-*E*) correlated with the sharp decrease in Q_B non-reducing centres (Fig. 3*C*-*E*) which was probably related to a certain neutralization of the negative effect of acid rain or to the activation of some protective mechanisms that metabolically ensure normal homeostasis of the plants.

Table 2. Influence of simulated acid rain of different pH on stomatal conductance, g_s [cm s^{-1}], transpiration rate, E [mg(H_2O) m^{-2} s^{-1}], and water use efficiency, WUE [g(CO_2) kg^{-1}(H_2O)]. Means ± SE of at least 3 experiments (5 measurements each). pH 5.6 means control. $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

[h]	g_s at pH 5.6	2.4	2.2	2.0	1.8	E 5.6	2.4	2.2	2.0	1.8	WUE 5.6	2.4	2.2	2.0	1.8
3	0.37±0.01	0.29±0.03	0.22±0.04*	0.20±0.02**	0.11±0.01***	64.0±8.2	55.2±4.9	49.8±7.9	47.3±6.6	27.9±3.5***	8.23	5.91	5.68	4.00	3.37
5	0.38±0.03	0.29±0.02**	0.28±0.03*	0.26±0.02**	0.20±0.02***	75.2±2.5	62.6±4.4*	60.3±6.1*	60.7±4.1**	48.5±4.7***	6.75	4.74	4.36	2.96	1.03
24	0.37±0.04	0.33±0.03	0.32±0.02	0.30±0.03	0.26±0.01**	60.0±4.4	60.6±4.8	59.3±4.0	60.1±4.3	52.0±3.1	8.22	6.65	6.34	3.33	3.19
48	0.36±0.03	0.30±0.02	0.33±0.03	0.32±0.03	0.30±0.03	65.9±4.4	58.5±4.4	64.6±4.2	69.0±6.3	61.4±4.1	7.81	7.09	5.85	4.40	3.81
72	0.26±0.01	0.27±0.02	0.29±0.01*	0.25±0.01	0.32±0.02**	48.8±2.3	52.7±4.7	55.8±2.9	53.8±2.4	61.9±3.2**	9.69	6.83	6.27	5.58	4.52
168	0.23±0.02	0.15±0.01**	0.17±0.02*	0.17±0.01**	0.19±0.01*	41.7±6.0	34.4±3.1	37.6±3.4	42.2±2.8	52.0±3.1	8.73	7.06	5.13	3.86	2.60

Discussion

Acid rain acts at the contact with leaves as well as through the soil acidification. The direct effect on leaves can lead to a decrease in assimilation area (Hogan 1992), changes in the cuticula (Barnes and Brown 1990), and stomata functioning. In consequence, changes in CO_2 uptake (Lechowicz 1987, McLaughlin 1988, Hogan 1992, Muthuchelian *et al.* 1995), water relations (Flückiger *et al.* 1988, Barnes *et al.* 1990a,b), and carbon metabolism (Hampp 1992) were observed. The increased acidity in chloroplasts can cause an injury of Chl proteins, especially of those included in PS2 (Siefermann-Harms 1992) and inhibit the rate of Calvin cycle enzymatic reactions (Woodrow *et al.* 1984).

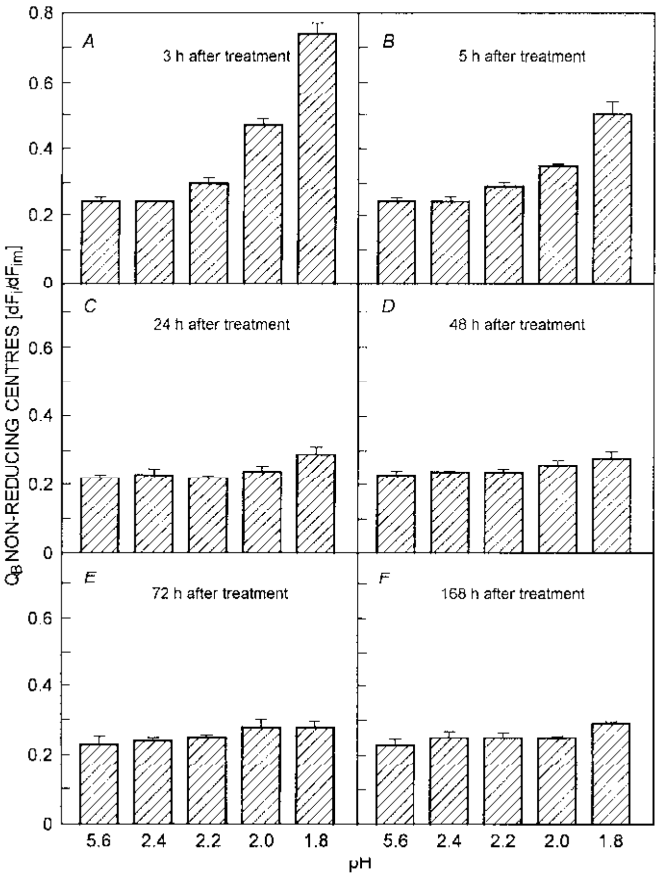

Fig. 3. Changes in relative concentration of Q_B-nonreducing photosystem 2 centres calculated from $[dF_i/dF_{im}] = (F_i-F_0)/(F_{im}-F_{i0})$ ratio after treatment of bean plants with simulated acid rain of different pH and duration. pH 5.6 means control.

At first sight, the pH values of simulated acid rain used in our experiment differed from those in the nature. Since the aim of our investigation was to establish the threshold pH value causing mild stress and irreversible injury of the photosynthetic apparatus, we applied acid rain with pH of 1.8-4.6. After single spraying of bean plants with this acid rain, a noticeable effect was observed only at pH values of 2.0 and 1.8. A single treatment of plants at different pH led to serious changes in P_N as well as in different parameters of PS2. A considerable suppression of P_N was established even in the first hours following acid treatment (Fig. 1*A,B*). A similar fast effect after SO_2 treatment was found in *P. vulgaris* (Oshima *et al.* 1973, Noyes 1980, Darrall 1986), *Vicia faba* (Black and Unsworth 1979), *Hordeum vulgare* (Taniyama 1972), *T. aestivum*, and *Brassica napus* (Darrall 1986). The period of a strong CO_2 uptake inhibition was followed by the period of recovery (Fig. 1*C-E*). Our results showed that pH values above 2.0 did not irreversibly damage the photosynthetic apparatus at a single spraying. This was probably related to a high buffer capacity of mesophyll cells, enabling the plants to reduce the unfavourable effect of low pH values. The damages of the photosynthetic apparatus resulting from treatments with pH 2.0 and 1.8 were on the limit of irreversible injuries and were the cause of a faster occurrence of ageing processes (Fig. 1*F*).

Simulated acid rain caused a decrease in g_s with a decrease in pH. This was probably connected with the reduced pressure potential of leaves. A similar change was observed after fumigation with ozone in beech seedlings (Mikkelsen 1995) and bush bean (Salam and Soja 1995). The combination of twice-ambient ozone and pH 3.0 rain lowered the seedling solute potential, turgor loss point and cell-wall elasticity in *Pinus ponderosa* (Momen and Helms 1995). The transpiration rate was reduced relatively less than P_N and this led to a lower WUE of the treated bean plants (Table 2).

The variable Chl *a* fluorescence is related to photochemical activity. Fluorescence increase from F_0 to F_m indicates plastoquinone photoreduction from PS2 (Bradbury and Baker 1981, Briantais *et al.* 1986, 1988). The established reduction of F_v (Figs. 2 and 3) might indicate the decreased ability of PS2 to reduce plastoquinone. One of the possible reasons for decreasing photochemical activity at a low pH could be related to the enhancement of relative concentration of the Q_B non-reducing $PS2_\beta$ centres. The breakdown of physical contact between the peripheral Chl-protein complexes and the PS2 core complex might induce, at a low pH, transitions of $PS2_\alpha$ to $PS2_\beta$, and their migration to unstacked membrane regions might reduce the transfer of excitation energy from LHC2 to the PS2 reaction centre (Goltsev *et al.* 1994).

Low pH values probably affect also the photophosphorylation (Ryrie and Jagendorf 1971) or the activity of Calvin cycle enzymes (Parry and Gutteridge 1984). After the plant treatment with simulated acid rain of a low pH, the activities of light reactions decreased less than P_N. The P_N is limited not only by light reactions but also by the decreased activity of Calvin cycle enzymes. Dizengremel *et al.* (1994) established a parallel decrease in ribulose-1,5-bisphosphate carboxylase/oxygenase activity and P_N during damages induced by O_3 in pine needles.

Our results showed that the inhibition of photosynthesis in plants treated with acid rain was due to a reduced photochemical activity, lowered activity of Calvin cycle enzymes, as well as to stomatal limitation. The period of a strong inhibition of CO_2 uptake and photochemical activity was followed by the period of photosynthetic functions´ restoration although to a different extent in treated plants (24-72 h). The decrease in P_N of plants treated with pH values of 2.0 and 1.8 after 168 h was probably due to an accelerated ageing.

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