

CHANGES IN PROMPT CHLOROPHYLL FLUORESCENCE AND OXYGEN EVOLUTION AFTER BEAN PLANT TREATMENT WITH ARTIFICIAL ACID RAIN

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Summary. The effects of artificially applied acid rain on functional activity of PS2 and oxygen evolution of bean plants (*Phaseolus vulgaris* L.) were investigated. The plants were subjected to single treatment with acid rain of different pH – pH 5.6 (control) and 2.4, 2.2, 2.0 and 1.8. Measurements were carried out in dynamics – at time intervals of 3–168 hours. The results showed that the treatment of plants with pH 2.4 and 2.2 did not change significantly initial (F_0) and variable (F_v) chlorophyll fluorescence as well as F_v/F_0 and F_v/F_m ratios characterizing the efficiency of PS2. Most important changes were established in plants sprayed with pH 1.8 cocktail. A similar trend was found in oxygen evolution measurements. It remained about 50% lower in plants treated with pH 1.8 in comparison with the control. We postulate that single treatment of bean plants with simulated acid rain caused considerable changes both in PS2 functional activity and oxygen evolution, but they were still reversible, although to a different extent.

Key words: acid rain, bean, chlorophyll *a* fluorescence, oxygen evolution, photosystem 2

Abbreviations: PS2 – photosystem 2; F_0 and F_v – initial and variable chlorophyll fluorescence; Q_A – primary electron acceptor of PS2

Introduction

Among atmospheric stress factors which reduce germination, growth and survival, acid rain is potentially one of great importance (Sheppard et al., 1993). Long-term exposure to acid rain can cause vegetation injury over large areas (Esher et al., 1992).

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Acid rain/mist can affect plants both through contact with the cuticle and via soil acidification. Direct contact of acid rain with leaves can cause significant impairment to assimilative organs (Hogan, 1992): first, it can alter the cuticular surface (Barnes and Brown, 1990) and the functioning of stomata. Consecutively, variations in cation leaching from foliage (Hogan, 1992), photosynthesis, water relations (Barnes et al., 1990), and carbon metabolism (Hampp, 1992) can also be found. Second, input of excess protons into stroma can depress the rate of enzymic reactions of Calvin's cycle (Woodrow et al., 1984), thus increasing sensitivity of the photosynthetic apparatus to photodamage (Osmond, 1981). Increased acidity in chloroplasts can also be harmful for chlorophyll-proteins, particularly those involved in PS2 (Siefermann-Harms, 1992).

In chloroplasts isolated from seedlings of tropical tree species a decrease in the activities of PS2 and whole electron transport chain was observed only at pH 3.0 and 2.0, but no significant change in PS1 activity was evident (Muthuchelian et al., 1995).

The results of Hogan and Taylor (1995) suggest that levels of acid precipitation from pH 3.0 to 5.5 did not act as a primary stress on physiological processes (gas exchange, chlorophyll *a* fluorescence). No significant interactive effects of simulated acid rain (pH 3.0 and 2.3) were observed on chlorophyll contents, carbon allocation and biomass accumulation of armand pine seedlings (Shan et al., 1995). The exposure of *Pinus armandi* seedlings to simulated acid rain (pH 2.3) reduced the net photosynthetic rate per unit chlorophyll *a+b* content, but did not induce a significant change in the other assessed parameters (transpiration rate, water-use efficiency and dark respiration rate) (Shan et al., 1996). A combination of twice-ambient ozone and pH 3.0 rain lowered *Pinus ponderosa* seedling solute potential, turgor loss point and cell-wall elasticity, but increased the pressure potential and simplistic water content, whereas total water content was unchanged (Momen and Helms, 1995). Chlorophyll fluorescence kinetics showed significant alterations following ozone treatment, reflecting a perturbation in the photochemical functioning of thylakoids, and specific disturbances of the water-splitting enzyme system of PS2 in *Vicia faba* (Guidi et al., 1993).

In this study, we present data about the effect of simulated acid rain of different pH on some chlorophyll fluorescence parameters and O₂ evolution in bean plants.

Materials and Methods

Plant material

Bean plants (*Phaseolus vulgaris* L. cv. Cheren Starozagorsky) were grown in 1.5 l glass vessels on Knop's nutrient solution in climatic chamber at 23–25 °C, photon flux density (PFD) 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 12/12 h day/night photoperiod. Each vessel was covered with a lid which did not permit acid rain to contaminate the nutrition solution. During the experiment its pH was controlled in order to verify if it changes

as a result of ion release from roots but such changes were not observed. Ten-day-old plants were separated into variants and sprayed only once. In preparing simulated acid rain the “cocktail” proposed by Seufert et al. (1990) was used. The plants were subjected to single treatment with acid rain of different pH – pH 5.6 (control) and 2.4, 2.2, 2.0 and 1.8. The measurements were carried out in dynamics – at time intervals of 3–168 hours.

Gas exchange and chlorophyll fluorescence measurements

The measurements were made on the 3, 5, 24, 48, 72 and 168th hour after treatment. The following parameters were measured on the primary leaves: a) chlorophyll fluorescence induction kinetics – by pulse modulated fluorometer PAM (H. Walz, Germany); b) O_2 evolution – by oxygen electrode LD2/2 (Hansatech, UK) on leaf discs (area 10 cm^2) at $25\text{--}28^\circ\text{C}$ and PFD $700\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Induction kinetics (IK) were registered and analyzed by computer programme FIP 4.3 (Tyystjarvi and Karunen, 1990).

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

Results and Discussion

Chlorophyll fluorescence

The major part of chlorophyll fluorescence of dark adapted leaves radiated at room temperature was that of chlorophyll *a* belonging to PS2 and light-harvesting complex 2 (LHC2) (Krause and Weis, 1991). *In vivo* measurements of chlorophyll fluorescence parameters made it possible to assess the photochemical capacity of PS2. Our previous data showed that single treatment of plants with different pH values of the cocktail is a reason for serious changes both in the rate of integral photosynthesis process (Velikova et al., 1996) and in the state of the thylakoid membranes evaluated by fluorescence parameters.

Fig. 1 shows the changes of the fluorescence parameters (F_0 and F_v) after acid treatments. Most important changes were established in plants sprayed with pH 1.8 cocktail. F_0 increased considerably (147%) already in the 3rd hour after spraying. Its value also remained higher at the 5th hour (122%) after which it gradually decreased to that of the control. Essential changes in F_0 were not registered in the rest of the variants. The increase of F_0 reflected the decrease of the transfer efficiency of the energy absorbed from the antenna chlorophyll *a* to the reaction centre of the PS2 and/or damages of the latter (Briantais et al., 1986). Schreiber and Armond (1978) suggest that the increase of F_0 caused by high temperature treatment leads most probably to

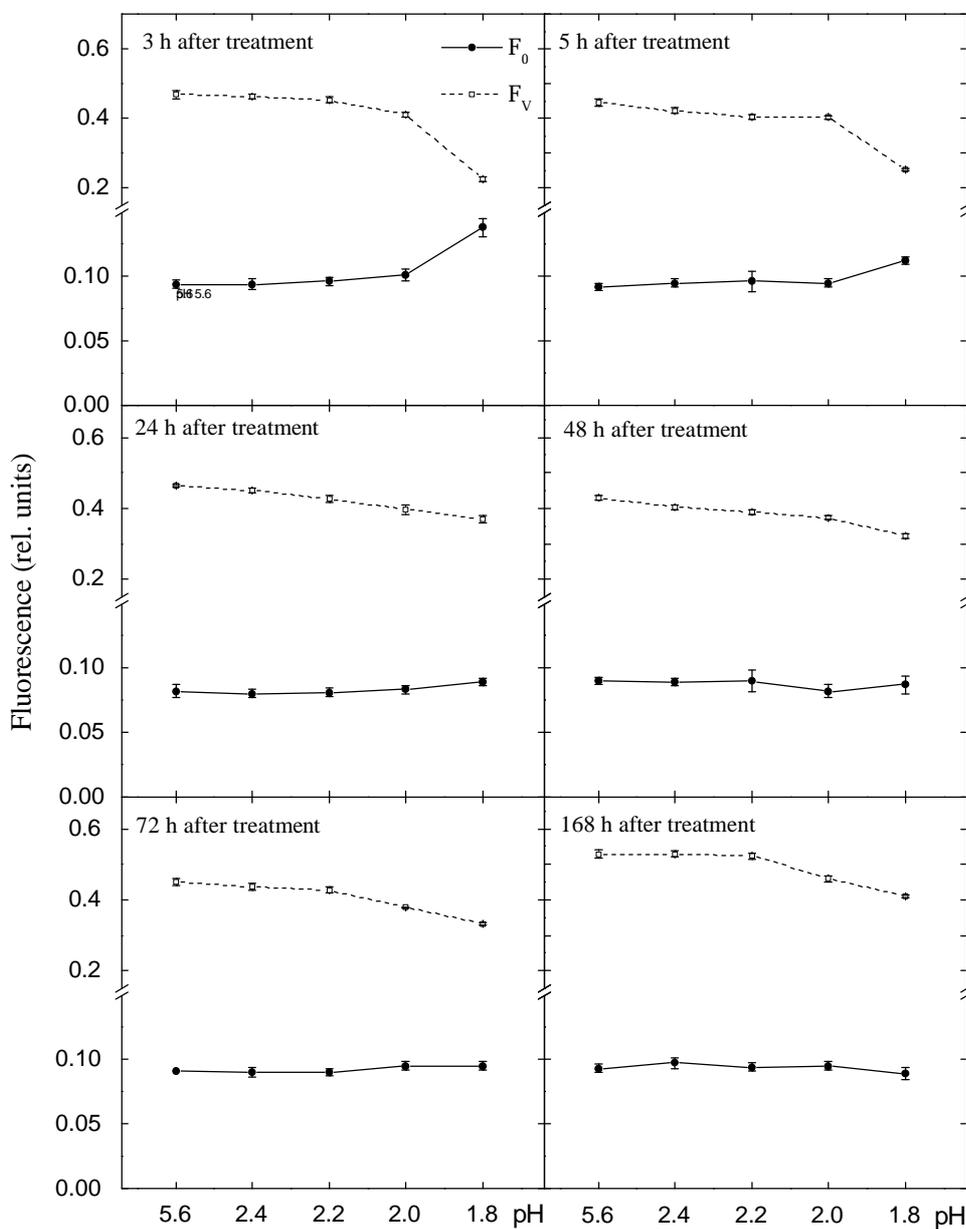


Fig. 1. Changes in F_0 and F_v parameters of chlorophyll *a* fluorescence of bean plants treated with simulated acid rain

dissociation of LHC from the RC of PS2, increasing in that way the F_0 level. The increase of F_0 in the first hours (3rd and 5th) after pH 1.8 acid treatment decreased and almost reached the reference values at the 168th hour. This provides a basis to

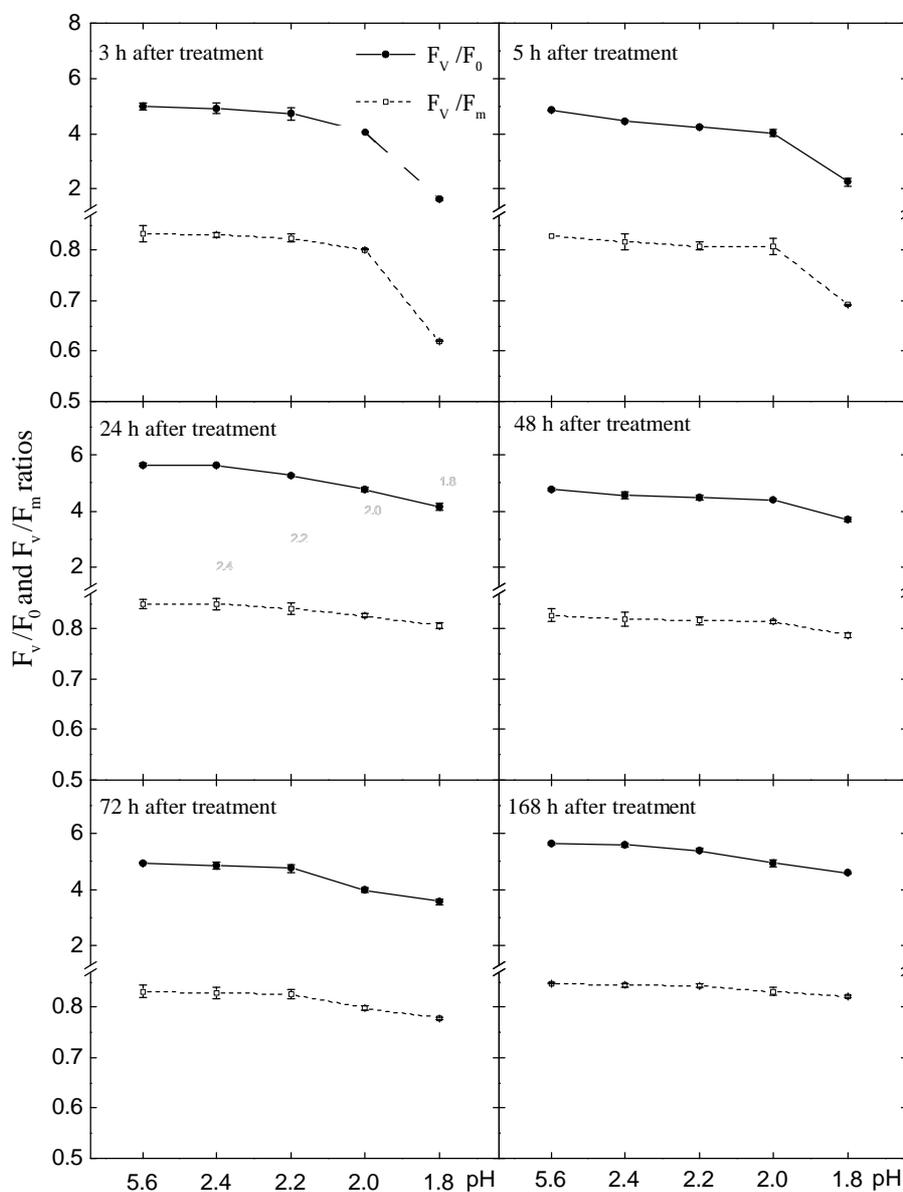


Fig. 2. Changes in F_v/F_0 and F_v/F_m ratios measured on leaf discs of treated bean plants with simulated acid rain

suggest that the conformation changes occurring in the thylakoid membrane and in the organisation of the pigment systems, provoking an increase of F_0 , are reversible.

At our experimental conditions (treatment with pH 1.8) changes in the reduced state of Q_A were the result of disbalance between the rates of Q_A reduction by PS2

and the rate of Q_A^- reoxidation by PS1. There are data indicating that Cu^{2+} treatment led to decrease in F_m and inhibited not only in the oxidising side of PS2 but also on P_{680} or in the reducing side of PS2 (Mohanty et al., 1989).

An important characteristic for PS2 activity is the variable fluorescence (F_v) which correlates with photochemical activity. Our results also showed important changes in respect to this component. Three hours after treatment by pH 1.8 F_v decreased to 48% and by pH 2.0 – to 88% of the control value. At the 5th hour in plants treated with pH 1.8 F_v represented 57% of the control (Fig. 1).

The decrease of F_v (which correlates with electron flow rate through the PS2) caused by simulated acid rain can be explained by the damage of the PS2 donor side (Bukhov et al., 1987). Bilger et al. (1985) found out that in isolated chloroplasts the decrease of F_v correlated with the degree of damage of the oxygen evolving system. The F_v decrease is interpreted as an indication for lowered PS2 capacity to reduce plastoquinone.

Results obtained show that F_0 and particularly F_v are informative characteristics for the processes of acidic injuries. They are in compliance with the available published data obtained under various stress effects: high temperature (Yordanov et al., 1997), photoinhibition (Stefanov et al., 1996), treatment by heavy metals (Krupa et al., 1993; Ouzounidou et al., 1995). The F_v/F_0 and F_v/F_m ratios are a measure for the PS2 efficiency in primary photochemical reactions. The stress factors affecting mainly the functional activity of this photosystem decreased these ratios (Krause and Weis, 1988). In plants treated with artificial acid rain at pH 1.8 on the 3rd hour the F_v/F_0 ratio drastically decreased (with more than 70%) compared to the control (Fig. 2), while in those treated by pH from 2.0 to 2.4 it decreased by about 20% during the whole time of investigation. Similar decrease of F_v/F_0 was reported for SO_2 fumigation of various plant species (Omasa et al., 1987) and in bean leaves treated with $NaHSO_3$ (Covello et al., 1989). In the author's opinion this obviously reflects the inhibition of PS2 activity (Schimazaki et al., 1984).

According to Björkman (1987) the F_v/F_m ratio is proportional to the quantum yield of the primary PS2 photochemical reaction. The value of F_v/F_m ratio is determined by the number of functioning reaction centres (Öquist et al., 1992). The results obtained show the same regularity in the change of F_v/F_m ratio as in F_v/F_0 (Fig. 2). The F_v/F_m ratio is lower in the leaves treated with pH 1.8 acid rain at the whole time of action. These changes are best expressed in the first hours (3rd and 5th), when F_v/F_m decreases to 60% and 70% of the control, respectively. In the rest of the variants no important deviations from the reference values are observed.

Oxygen evolution

Data for oxygen evolution after the action of acid rain are presented in Fig. 3. Three hours after treatment by pH 1.8 a strong decrease (by 70%) of this parameter was

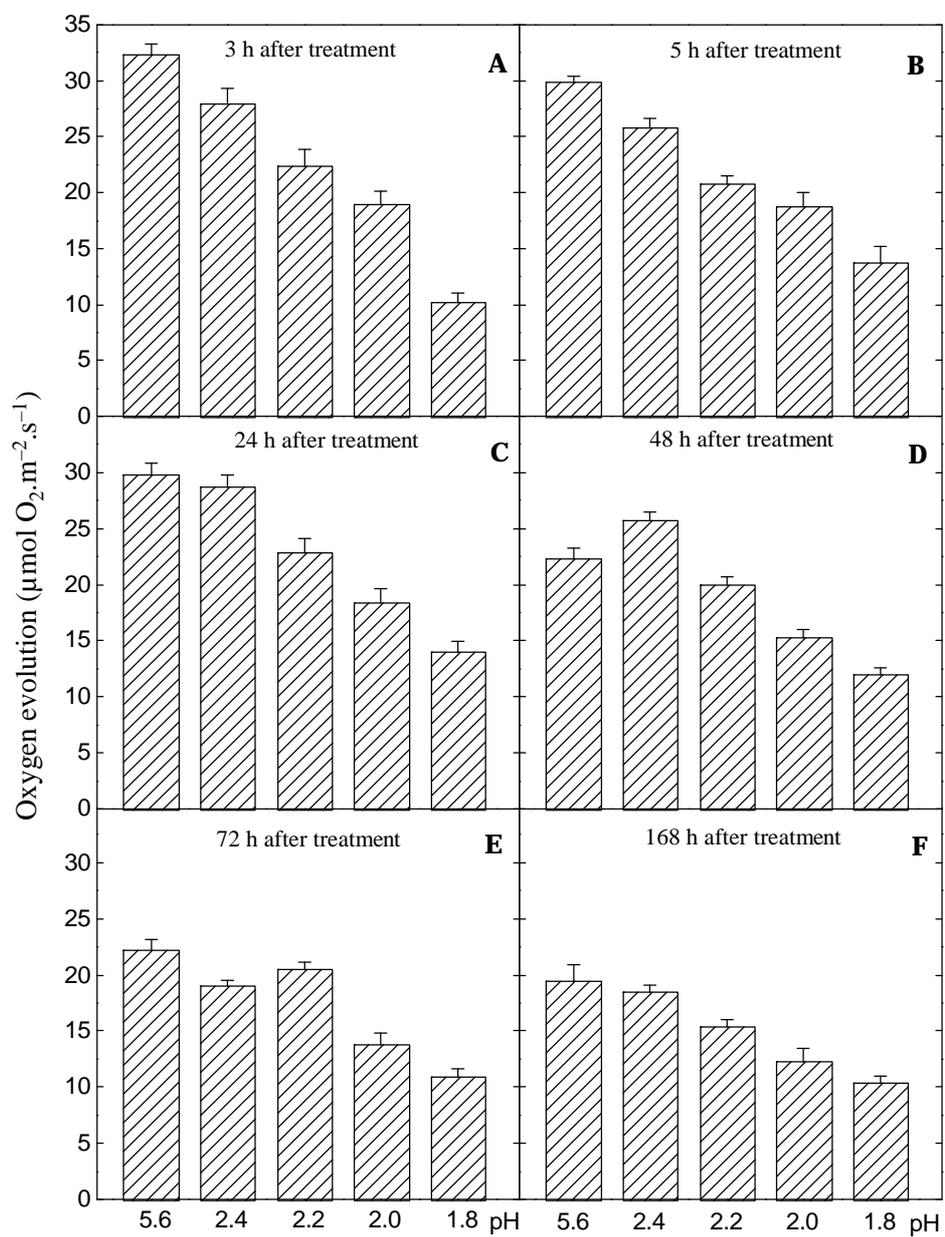


Fig. 3. Influence of simulated acid rain with different pH on oxygen evolution measured on bean discs

observed. A similar trend was found in the rest of the variants (pH 2.4, 2.2 and 2.0). Oxygen evolution in plants treated with acid rain of different pH decreased as follows: 14% (pH 2.4), 31% (pH 2.2) and 42% (pH 2.0). The degree of oxygen inhibition was also preserved 5 h after treatment, excluding the variant sprayed with cocktail of pH 1.8. These results showed that single spraying of bean plants with simulated acid rain of low pH values (pH 2.0 and 1.8) did not lead to unreversible unfavourable changes in the oxygen evolving system. Results in subsequent measurements (24–168 h) support our supposition. All treated plants showed a tendency, though to a different extent, enhancing their oxygen evolving capacity. For example, oxygen evolution of plants sprayed with acid rain of pH 2.4 reached almost the control value (Fig. 3C) on the 24th hour and on 48th hour it was even higher (115%) than the control one (Fig. 3D). Therefore, acid rain of pH 2.4 applied singly did not lead to substantial changes in oxygen evolution. Similar dependence was observed in plants treated with acid rain of pH 2.2. The degree of oxygen evolution recovery reached about 90% of control value after 48th and 72nd hours (Fig. 3C, D, E). A tendency to recovery was observed even in plants treated with pH 2.0 and 1.8, but this process proceeded very slowly. After spraying of plants with pH 2.0 the oxygen evolution recovered to about 60% of the control (Fig. 3B-F). Naturally, the lowest ability to recover oxygen evolving activity, about 50% of the control, was observed in heavily injured plants treated with acid rain of pH 1.8.

It is obvious from these results that changes registered at the 3rd hour after treatment by various pH values are the most important. Measurements in dynamics show some recovery of the oxygen evolution in the plants treated with pH 2.4 while in the other variants the measurements indicate its prolonged inhibition. In plants sprayed with pH 1.8 oxygen evolution remained about 50% below the control, but at all time intervals of measurement it was higher than the oxygen release at the 3rd hour, i.e. some recovery could be observed.

Single treatment by different pH values of the cocktail is the reason for considerable changes both in the rate of the integral photosynthetic process and in the state of the thylakoid membranes as well as in the parameters of chlorophyll *a* fluorescence, characterising the PS2 functional activity. These changes are related with the decrease of PS2 efficiency in the first photochemical reactions (decrease of the F_v/F_0 and F_v/F_m ratios). The comparison of the data concerning oxygen evolution and chlorophyll fluorescence, which characterize the influence of acid rain with different pH on the functional activity of the photosynthetic apparatus showed that the oxygen evolving system is definitely more affected and the degree of its recovery is lower in comparison with the fluorescence parameters investigated.

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References

- Barnes, J. D., K. A. Brown, 1990. The influence of ozone and acid mist on the amount and wettability of the surface waxes in Norway spruce [*Picea abies* (L.) Karst.]. *New Phytol.*, 114, 531–535.
- Barnes, J. D., D. Eamus, K. A. Brown, 1990. The influence of ozone acid mist and soil nutrient status on Norway spruce [*Picea abies* (L.) Karst.]. II. Photosynthesis, dark respiration and soluble carbohydrates of trees during late autumn. *New Phytol.*, 115, 149–156.
- Bilger, W., U. Schreiber, O. L. Lange, 1985. Chlorophyll fluorescence as an indicator of heat induced limitation of photosynthesis in *Arbutus unedo*. NATO Advanced Research Workshop Sesimbra, Portugal, October.
- Björkman, O., 1987. Low-temperature chlorophyll fluorescence in leaves and its relationship to photon yield of photosynthesis in photoinhibition. In: *Photoinhibition*, Eds. D. J. Kyle, C. B. Osmond and C. J. Arntzen, Amsterdam, Elsevier, pp. 123–144.
- Briantais, J.-M., C. Vernotte, G. H. Krause, E. Weis, 1986. Chlorophyll *a* fluorescence of higher plants: chloroplasts and leaves. In: *Light Emission by Plant and Bacteria*, Eds. Govindjee, J. Amesz and D. J. Fork, New York, Academic Press, pp. 539–583.
- Bukhov, N. G., T. G. Djibladze, N. V. Karapetyan, 1987. Influence of high temperatures on the kinetics of variable and delayed fluorescence. *Plant Physiol. (Moscow)*, 34(3), 435–444 (In Russ.).
- Covello, P. S., A. Chang, E. B. Dumbroff, J. E. Thompson, 1989. Inhibition of photosystem II precedes thylakoid membrane lipid peroxidation in bisulfite treated leaves of *Phaseolus vulgaris*. *Plant Physiol.*, 90, 1492–1497.
- Esher, R. J., D. H. Marx, S. J. Baker, L. R. Brown, D. C. Coleman, 1992. Simulated acid rain impact on fine roots, endomycorrhizae, microorganisms, and invertebrates in pine forest of the southern United States. *Water, Air and Soil Poll.*, 61, 269–278.
- Guidi, L., A. Panicucci, G. Lorenzini, G. F. Soldatini, 1993. Ozone induced changes in chlorophyll fluorescence kinetics and CO₂ assimilation in *Vicia faba*. *J. Plant Physiol.*, 141, 545–550.
- Hampp, R., 1992. Comparative evaluation of the effects of gaseous pollutants, acidic deposition and mineral deficiencies on the carbohydrate metabolism of trees. *Agriculture, Ecosystems and Environ.*, 42, 333–364.
- Hogan, G. D., 1992. Physiological effects of direct impact of acidic deposition on foliage. *Agriculture, Ecosystems and Environ.*, 42, 307–319.
- Hogan, G. D., S. J. Taylor, 1995. Effect of acidic deposition on hybrid poplar – primary or predisposing stress. *Water, Air and Soil Pollution*, 85, 1419–1424.
- Krause, G. H., E. Weis, 1988. The photosynthetic apparatus and chlorophyll fluorescence. An introduction. In: *Application of Chlorophyll Fluorescence*, Ed. H. Lichtenthaler, Dordrecht, Kluwer Press, pp. 3–11.
- Krause, G. H., E. Weis, 1991. Chlorophyll fluorescence and photosynthesis. The Basics. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 42, 313–349.

- Krupa, Z., G. Öquist, N. Hunner, 1993. Chlorophyll fluorescence and photoacoustic characteristics in relationship to changes in chlorophyll and Ca^{2+} content of a Cu-tolerant *Silene compacta* ecotype under Cu treatment. *Physiol. Plant.*, 88, 626–630.
- Mohanty, N., I. Vass, S. Demeter, 1989. Copper toxicity affects photosystem II electron transport at the secondary quinone acceptor, Q_B . *Plant Physiol.*, 90, 175–179.
- Momen, B., J. A. Helms, 1995. Osmotic adjustment induced by elevated ozone-interactive effects of acid-rain and ozone on water relations of field-grown seedlings and mature trees of *Pinus ponderosa*. *Tree Physiology*, 15, 799–805.
- Muthuchelian, K., C. Murugan, R. Harigovindan, N. Nedunchezian, G. Kulandaivelu, 1995. Growth, CO_2 - C^{14} fixation, activities of photosystems, ribulose 1,5-bisphosphate carboxylase and nitrate reductase in trees as affected by simulated acid rain. *Biol. Plantarum*, 37(3), 355–362.
- Omasa, K., K. Shimazaki, I. Aiga, W. Larcher, M. Onoe, 1987. Image analysis of chlorophyll fluorescence transients for diagnosing the photosynthetic system of attached leaves. *Plant Physiol.*, 84, 748–752.
- Osmond, C. B., 1981. Photorespiration and photoinhibition. Some implications for the energetic of photosynthesis. *Biochim. Biophys. Acta*, 639, 77–89.
- Ouzounidou, G., M. Moustakas, R. Lannoye, 1995. Chlorophyll fluorescence and photoacoustic characteristics in relationship to changes in chlorophyll and Ca^{2+} content of a Cu-tolerant *Silene compacta* ecotype under Cu treatment. *Physiol. Plant.*, 93, 551–557.
- Öquist, G., W. S. Chow, J. M. Anderson, 1992. Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosynthesis. *Planta*, 186, 450–460.
- Schreiber, U., P. A. Armond, 1978. Heat-induced changes of chlorophyll fluorescence in isolated chloroplasts and related heat-damage at the pigment level. *Biochim. Biophys. Acta*, 502, 138–151.
- Seufert, G., V. Hoyer, H. Wöllmer, U. Arndt, 1990. The Hohenheim long-term experiment. General methods and materials. *Environ. Pollut.*, 68, 205–229.
- Shan, Y., Z. Feng, T. Izuta, M. Aoki, T. Totsuka, 1995. The individual and combined effects of ozone and simulated acid-rain on chlorophyll contents, carbon allocation and biomass accumulation of armand pine seedlings. *Water, Air and Soil Pollut.*, 85, 1399–1404.
- Shan, Y. F., Z. W. Feng, T. Izuta, M. Aoki, T. Totsuka, 1996. The individual and combined effects of ozone and simulated acid-rain on growth, gas-exchange rate and water-use efficiency of *Pinus armandi* franch. *Environ. Pollut.*, 91, 355–361.
- Sheppard, L. J., J. N. Cape, I. D. Leith, 1993. Influence of acidic mist on frost hardiness and nutrient concentrations in red spruce seedlings. 1. Exposure of the foliage and the rooting environment. *New Phytol.*, 124, 595–605.
- Shimazaki, K., K. Nakamachi, N. Kondo, K. Sugahara, 1984. Sulfite inhibition of photosystem II in illuminated spinach leaves. *Plant Cell Physiol.*, 25, 337–341.
- Siefermann-Harms, D., 1992. The yellowing of spruce in polluted atmosphere. *Photosynthetica*, 27, 323–341.

- Stefanov, D., I. Yordanov, T. Tsonev, 1996. Effect of thermal stress combined with different irradiance on some photosynthetic characteristics of barley (*Hordeum vulgare* L.) plants. *Photosynthetica*, 32(2), 171–180.
- Tyystjarvi, E., J. Karunen, 1990. A microcomputer program and fast analog to digital converter card for the analysis of fluorescence induction transients. *Photosynth. Res.*, 26, 127–132.
- Velikova, V., I. Yordanov, M. Kurteva, 1996. Photosynthetic characteristics of *Phaseolus vulgaris* plants treated with simulated acid rain with different pH and duration. *Comp. rend Acad. bulg. Sci.*, 49(12), (in press).
- Woodrow, I. E., D. J. Murphy, E. Latzko, 1984. Regulation of stromal sedoheptulose-1,7-bisphosphatase activity by pH and Mg^{2+} concentration. *J. Biol. Chem.*, 259, 3791–3795.
- Yordanov, I., T. Tsonev, V. Goltsev, M. Merakchiiska-Nikolova, K. Georgieva, 1997. Gas exchange and chlorophyll fluorescence during water and high temperature stress and recovery. Probable protective effect of carbamide cytokinin 4-PU30. *Photosynthetica*, 33(2), (in press)