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EFFECTS OF PROLONGED ACTION OF SUB-HERBICIDE CONCENTRATIONS OF ATRAZINE ON THE PHOTOSYNTHETIC FUNCTION OF PEA PLANTS

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Abstract

The effect of low atrazine concentrations on young pea plants was monitored during their development by means of fluorescence induction analysis. It was shown that atrazine in concentrations compatible to those found in underground and surface water has a significant gradually increasing impact on the photosynthetic function.
Key words: herbicide residuals, atrazine, chlorophyll fluorescence

Introduction. Triazines are one of the economically most important and widely used groups of selective herbicides. The main representative of this group is atrazine (2-chloro-4-isopropyl-6-ethyl-*s*-triazine). It is well known that the extensive agricultural use of any herbicides has caused their wide accumulation in underground and surface water and soil, as well as their distribution by aerosols [1,2]. Unfortunately, limited information is available about the post-effects of atrazine and other *s*-triazines in relation to their main targets – the plant species susceptible to their action. The herbicide efficiency is most commonly judged by the I_{50} plant growth inhibition [3]. The effect of herbicide residuals on the productivity of crops grown on areas previously treated with atrazine was scarcely studied [4].

Photosynthesis is the primary target of the triazines. Atrazine blocks the photoinduced electron transport by specific binding to the Q_B-site of the D1 protein in the Photosystem 2 (PS 2) reaction centre [5,6]. A popular non-destructive probe of the functional state of the photosynthetic apparatus is the chlorophyll fluorescence [7,8]. Direct registration of fluorescence induction transients with high time resolution has been used to screen the fate of the excitation energy within the photosynthetic apparatus and the electron transport through PS 2 [9].

Materials and methods. As a model system pea (*Pisum sativum* L., cv. Manuela) plants were used. Seeds were soaked on tap water for 4–6 h and put on moisturised filter paper in Petri dishes for germination (25 °C, in the dark, 72 h). Seedlings were grown as a water culture (Hoagland-Arnon nutrient medium, changed every

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2 days; growth chamber conditions – 12/12 h day/night photoperiod, light intensity ca. 60 μmol.m^{-2}.s^{-1}; temperature 25 ± 2 °C). Atrazine was added to the nutrient solution in concentrations of 10^{-7} M (0.022 mg/l), 10^{-6} M or 10^{-5} M. Chlorophyll fluorescence induction curves were registered 7, 14 and 21 days after the herbicide application. Measurements were made on detached leaves, dark-adapted for 3 min, using HandyPEA fluorometer (Hansatech, UK) for a period of 5 s at a maximal time resolution of 10 μs. Actinic light intensity was 3000 μmol.m^{-2}.s^{-1}. Ten leaves of each experimental group were sampled.

Fluorescence parameters were calculated according to STRASSER et al. [9] by using the Biolyzer software, kindly provided by the author, Ronald Maldonado-Rodriguez. The parameters ABS/CS_M, TR_0/CS_M, ET_0/CS_M and DI_0/CS_M represent the energy fluxes through PS 2 calculated per leaf area (excited cross section). $ABS/CS_M = F_M$ is used as a measure of the absorption energy flux; $TR_0/CS_M = F_M - F_0$ is the initial (maximal) flux of photochemically conserved (trapped) excitation energy; $ET_0/CS_M = F_M - F_J$ represents the flux of electrons transferred between the two photosystems, and $DI_0 = F_0$ is a measure of the energy dissipated as heat or fluorescence. As general indicators of the photosynthetic activity the parameters performance index (PI) and driving force (DF) were used as defined in [9], remarks.

Results and discussion. The fluorescence induction parameters (PI) and (DF) registered from pea plants grown at different atrazine concentrations for 7, 14 and 21 days, respectively, are presented in Fig. 1. The results show that photosynthesis

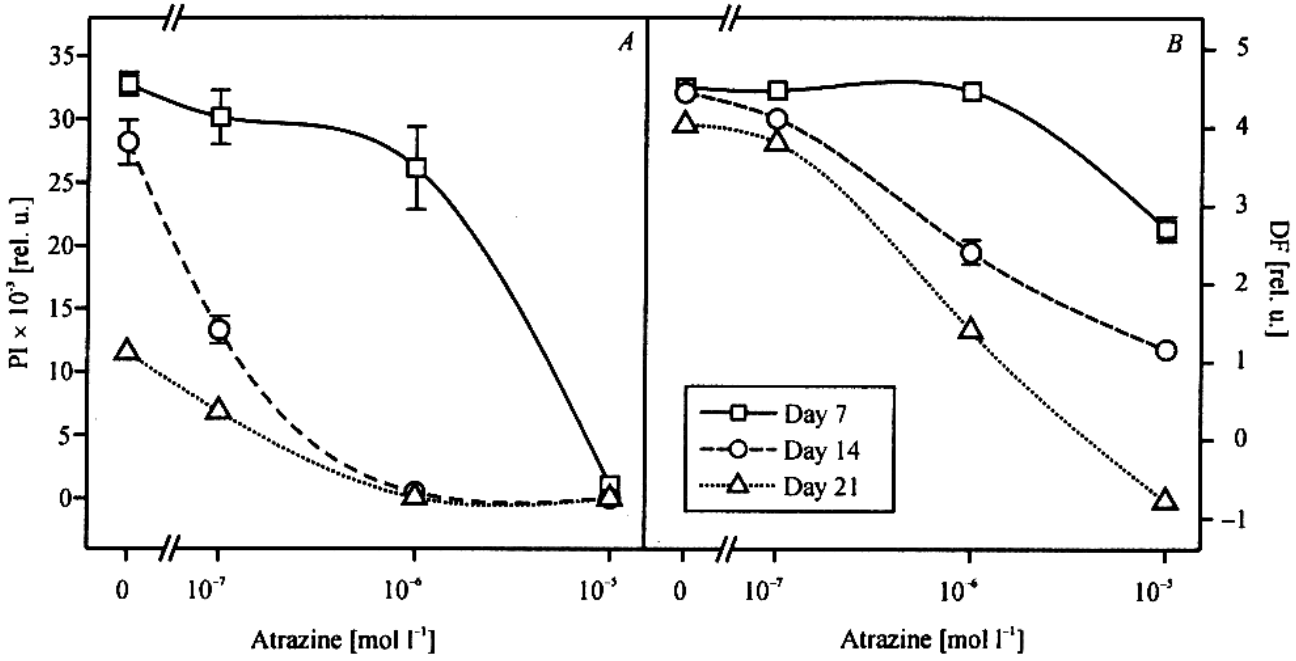

Fig. 1. Concentration dependence of the chlorophyll fluorescence induction parameters PI(CS_M) (*A*) and DF (*B*) of pea plants grown for 7, 14 or 21 days on a medium containing atrazine. Fluorescence induction curves were registered from 3-min dark-adapted detached second leaves by using HandyPEA fluorometer at actinic light intensity 3000 μmol.m^{-2}.s^{-1}. The vertical bars represent standard errors

of atrazine-treated plants was progressively inhibited within the time. The herbicide effect reached its maximum after 14 days. The fluorescence parameters had even lower absolute values on the 21st day, however, the difference with the control was smaller since PI and DF decreased in the control samples. This could be explained by the natural senescence processes of the second leaf which normally occurred approximately

three weeks after germination. Plants grown on 10^{-7} M atrazine-containing medium were not statistically different from the control after 7 days but a strong inhibition was evident on the 14th day.

The herbicide effect on the functional state of the plant can be evaluated differentially by analysing the JIP parameters, related to specific energetic fluxes through PS 2. The effect of 10^{-6} M atrazine on the parameters related to the absorption of excitation energy (ABS/CS_M), photochemical trapping (TR_0/CS_M), electron transport (ET_0/CS_M) and energy dissipation (DI_0/CS_M) in the initial moment of induction, registered on the 14th day of herbicide treatment is presented in Fig. 2. While the effect

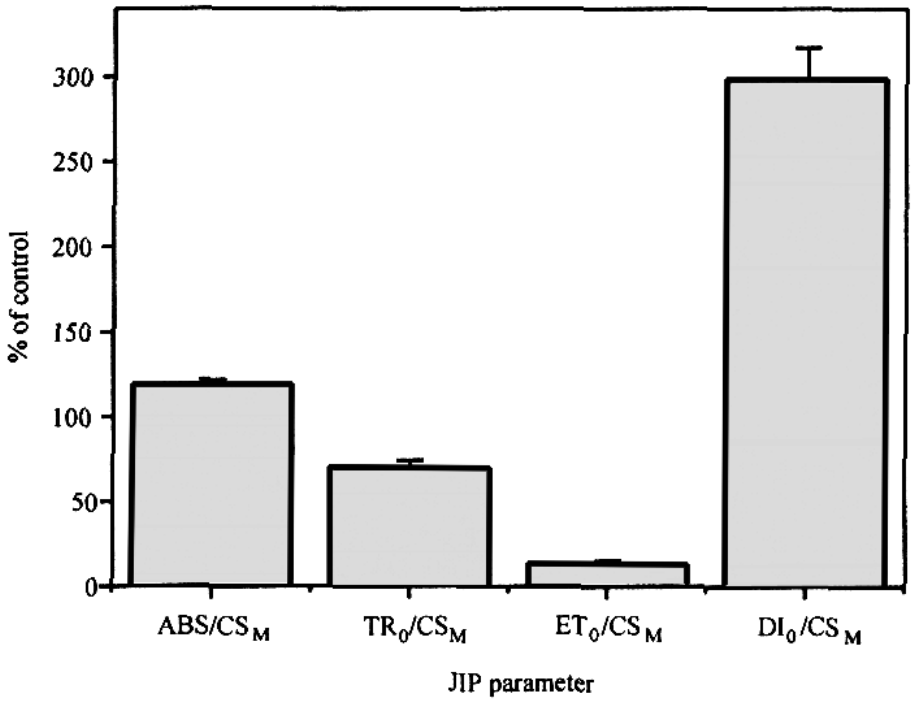

Fig. 2. Effect of 10^{-6} M atrazine present in the growth medium of 14-days old pea plants on the JIP parameters ABS/CS_M, TR_0/CS_M, ET_0/CS_M and DI_0/CS_M. Experimental conditions were as in Fig. 1. Values are normalized to the control. Vertical bars represent standard errors

on ABS and TR_0 was relatively small (120% and 70% of the control, respectively), electron transport (ET_0/CS_M) was inhibited by nearly 90% and the dissipated energy increased 3-fold compared to the control.

Thus, the presence of atrazine directly alters the photosynthetic state of the plant. The straight herbicide effect is well studied and easily detected by chlorophyll fluorescence in vivo when atrazine is applied at high concentrations [10].

Prolonged atrazine treatment during the plant development had a significant effect on photosynthesis which gradually increased with time. To explain this observation it could be supposed that atrazine concentration inside the chloroplasts increases during the plant growth. The registered effect on photosynthetic parameters would then be proportional to the fraction of blocked reaction centres during registration. On the other hand, the fluorescence changes could reflect the physiological response to the continuous state of partial inhibition of photosynthesis, i.e. the herbicide "post-effect". Treatment of plants with 10^{-6} M herbicide for two weeks provoked a decrease in fresh weight. Similar results were obtained after the application of 10^{-7} M atrazine for three weeks (unpublished results). The blocking of PS 2 by atrazine usually implicates oxidative

stress [11,12]. However, preliminary results in the same model system showed that there were no significant changes in the levels of oxidative stress markers (unpublished data).

By comparing the JIP-test parameters we found that the herbicide effect is mainly due to inhibition of the electron transport in the acceptor side of PS 2. The absorption of light energy per reaction centre (PS 2 antenna size) was stimulated in atrazine-treated plants as indicated by the parameter ABS/CS_M. This is in accordance with the work of JOSHI et al. [13], who have showed that prolonged partial inhibition of PS 2 in pea plants leads to increase of the photosynthetic unit size. However, when electron transport is blocked, a large portion of the excitation energy is dissipated as heat and fluorescence (DI_0/CS_M).

Conclusion. Our investigations showed that low concentrations of atrazine which are close to the residuals found in underground and surface water disrupted the normal photosynthetic function of pea plants and the effect observed was amplified with time. The chlorophyll fluorescence method proved to have the relevant sensitivity to reveal the effects of weak xenobiotic presence.

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