THE EFFECT OF TRIAZINE- AND UREA-TYPE HERBICIDES ON PHOTOSYNTHETIC APPARATUS IN CUCUMBER LEAVES

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ABSTRACT

About a half of the herbicides used at present in agriculture inhibit the light reactions in photosynthesis. Triazines and phenylureas shut down the photosynthetic process in susceptible plants by binding to specific sites within the plants photosystem II (PS II) complex. Both of them bind at the QB site on the D1 protein of PS II, and prevent the transport of electrons between the primary electron acceptor Q and the plastoquinone (PQ). Herbicides can be highly toxic to human and animal health (triazines are possible human carcinogens). Their indiscriminate use has serious environmental implications, for example pollution of soil and water. We compare two herbicides to investigate the one of lowest environmental toxicity but of high toxicity to weeds

KEY WORDS: photosystem II, chlorophyll fluorescence, cucumber, herbicide, simazine, linuron, net photosynthetic rate, toxicity.

INTRODUCTION

Photosynthetic organisms can directly utilize the energy of sunlight for synthesis of organic compounds. In higher organisms, all the molecular complexes involved in energy conversion and CO_2 fixation are concentrated in chloroplasts. CO_2 fixation takes place in the chloroplast stroma but light reactions, on the other hands, are almost exclusively confined to the membranes. The majority of the tylakoid membrane proteins are represented by number of multisubunit complexes. The key to the photosynthetic electron transport chain is the presence of two large complexes known as photosystem I (PSI) and photosystem II (PSII) (Hopkins and Hüner 2004). PS II complex converts light energy into chemical form using water as a source of electrons and liberating molecular oxygen as a side product. From the enzymatic point of view PSII is water/plastoquinone oxidoreductase. The structure of the PSII reaction center was proposed to have a two-fold symmetry with D1 and D2 forming skeleton and with CP43 and CP47 as chlorophyll-binding proteins. In addition to their structural role, some of the amino acid residues of hydrophobic subunits D1 are directly involved in electron transport and in charge separation (Svensson et al.1996; Hankamer et al 1997; Svensson et al.1999).

In a number of papers (Ohad and Hirschberg 1990; Sobolev and Edelman 1991; de Wijn and van Gorkom 2001), D1 is referred to as the Qb-protein or herbicide binding protein because D1 contains the binding site for plastoquinone (Qb) and the herbicides, too. The Qb binding niche is formed by amino acids from the end of transmembrane helix D, the beginning of transmembrane helix E, and hydrophilic loop exposed to the stroma and connecting helices D and E. The quinone is crosslinks with hydroxy group of Ser264 and the nitrogen of His215 (Kless et al. 1994; Praczyk and Skrzypczak 2004).

A number of commercially available herbicides, including triazines (simazine) and derivatives of urea (linuron) inhibit photosynthetic electron flow via a direct interaction with the Qb-protein. Different residues in the Qb niche are involved in binding different inhibitors. Triazine and urea type herbicides are proposed to be oriented towards Ser264 and phenols towards His215, both amino acids are located in the region between helices D and E. The herbicide interferes with the binding of plastoquinone to the same site and thus block the transfer of electrons to plastoquinone. (Praczyk and Skrzypczak 2004).

On the other hand, a conformational change of the Qb-niche after the herbicide binding inhibits degradation of the D1 protein. This may indicate a central role of the Qb niche in the life cycle of the D1 protein (Krieger-Liszkay and Rutherford 1998).

List of abbreviations

PS II – complex of photosystem II; Qb – plastoquinone; PN – net photosynthetic rate; Fv/Fm – potential efficiency of PS II; ΦPS2 – actual quantum efficiency of PS II; GS – stomatal conductance; qP – photochemical quenching fluorescence of chlorophyll; qNP – non-photochemical quenching fluorescence of chlorophyll; DM – dry mass

In this paper we compare the effect of two herbicides (simazine and linuron) on light reactions of photosynthesis in cucumber seedlings.

MATERIAL AND METHODS

Cucumber (*Cucumis sativus* L. var. Krak1) seeds germinated in darkness at 25°C were cultivated hydroponically in Hoagland solution 3 times diluted (Buczek and Marciniak 1990). After 10 days seedlings were transferred to the fresh nutrient solutions which contained herbicides (except control): simazine at concentration 0.05; 0.5; 5 mg/dcm^3 or linuron at concentration 0.045; 0.45; 4.5 mg/dcm^3. The method of treating of plants with herbicides used here was adapted after preliminary experiments using various herbicide concentrations and times of exposure of seedlings. Plants were grown under 16 h photoperiod (180 µmol.m^{-2} s^{-1}) at 25°C during the day and 22°C during the night. After next 1 or 3 days fully expanded first leaf each of seedling was used for the analyses.

Chlorophyll fluorescence was measured with a pulse amplitude modulated system (model FMS2, Hansatech instruments, UK). Prior to the analyses, the leaves were dark adapted for 20 min in order to obtain the potential fluorescence (Fv/Fm). After the dark period, all centers of PSII were "open", i.e. their electron acceptors Q_A were oxidized. The actual quantum efficiency of PSII (ΦPS2) was determined after adaptation of leaves to an actinic "white light" (600 µmol/m^{-2}/s^{-1}), when steady state of the fluorescence yield was reached.

Net photosynthetic rate (PN) and stomata conductance (GS) were measured with portable photosynthesis measuring system CIRAS –1 (*PP*Systems, Hitchin, U.K.) equiped with broad leaf cuvette, at irradiance of 300 µmol/m^{-2}/s^{-1}, 360 cm^3 CO_2 in air, and leaf temperature of 25°C.

Plants used for analysis were then removed from nutrient solution, dried at 70°C for five days, weighting and dry mass was determined.

Chemicals used: simazine (6-chloro-N^2,N^4-diethyl-1,3,5--triazine-2,4-diamine), linuron (3-(3,4-dichlorophenyl)-1--methoxy-1-methylurea). These chemicals were reagent grade and used without further purification.

The standard deviations (SE) were calculated for ten replications from three independent experiments. Differences which exceeded twice the standard error were considered to be significant at the 5 per cent level. Gas exchange and fluorescence parameter measurements were performed on ten individual plants per treatment.

RESULTS

Figure 1 shows the influence of simazine and linuron on the net photosynthesis rate (PN) of cucumber seedlings cultivated at the presence of herbicides. Inclusion of simazine into the nutrient solution at concentration 0.05; 0.5; 5 mg/dcm^3 respectively, decreased PN after 1 day by 30% at 0.5 mg/dcm^3 and by 100% at 5 mg/dcm^3. At concentration 0.05 mg/dcm^3 of simazine no inhibition was observed. The treatment of seedlings with simazine for 3 days clearly lowered photosynthetic absorption of CO_2. At concentrations of the herbicide: 0.5 mg/dcm^3 and 5 mg/dcm^3 respectively, we observed predominance of respiration. Simazine at 0.05 mg/dcm^3 did not affect significantly the rate of photosynthesis net (PN), whereas the introduction of linuron into the nutrient solution strong decreased rate of photosynthesis by 30%, 60%, 100% respectively after 1 day of treatment, and by 50%, 100%, 100% respectively after 3 days.

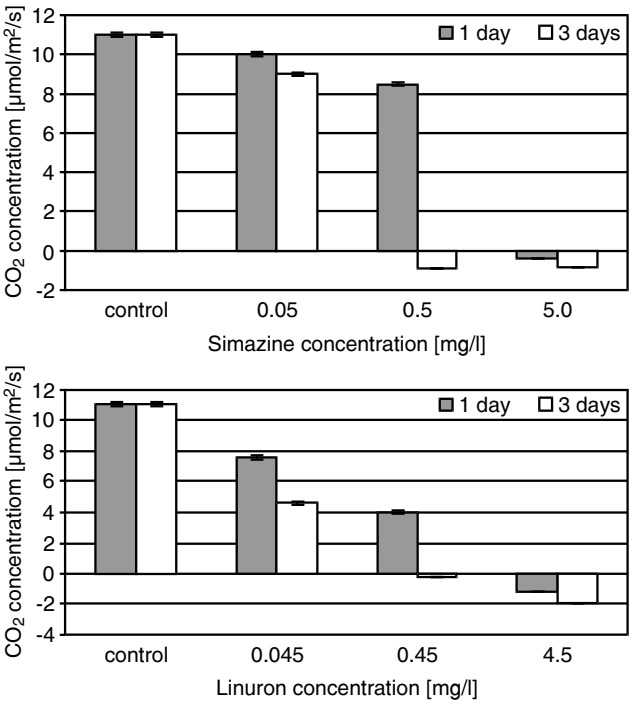

Fig. 1. Net photosynthesis rate for cucumber seedlings cultivated at the presence of simazine or linuron. The net photosynthesis rate (PN) for control plants was about 11.0 µmol CO_2/m^2/s^1. Inclusion of simazine at concentration 0.05; 0.5; 5 mg/l into the nutrient solution decreased PN after 1 day by 30% at 0.5 mg/l and by 100% at 5 mg/l. After 3 days we observed predominance respiration at both concentration. At 0.05 mg/l no inhibition was observed. Inclusion of linuron at concentration 0.045; 0.45; 4.5 mg/l drastically decreased PN: 30%, 60% and 100% respectively after 1 day and 50%, 100%, 100% after 3 days.

Figure 2 presents the variable fluorescence parameter (Fv/Fm) of photosynthesis. The plants grew for 1 or 3 days preceding assays in herbicides containing nutrient solution. Potential efficiency of photosystem II (Fv/Fm) was determined after 20 min. dark adaptation of seedlings leaves. The ratio of Fv/Fm independently of time exposition to herbicide was reduced similarly, for both simazine and linuron, compare with the control.

Over the same range of herbicides concentrations, the actual photosystem II quantum efficiency (ΦPS2) was more inhibited at linuron (50%, 80%, 100% respectively) to compare with simazine (0%, 75%, 85% respectively) (data presented at Figure 3). The continuation of growth of cucumber seedlings for 3 days under the same conditions was correlated with further decrease in the actual PSII efficiency (Fig. 4)

A comparison between amount of electrons used for light (photochemical) reaction of photosynthesis or for fluorescence (non photochemical reaction) are presented in Figure 4. Simazine as well as linuron decreased the level of photochemical (qP) and non photochemical (qNP) flow of electrons through photosystem II.

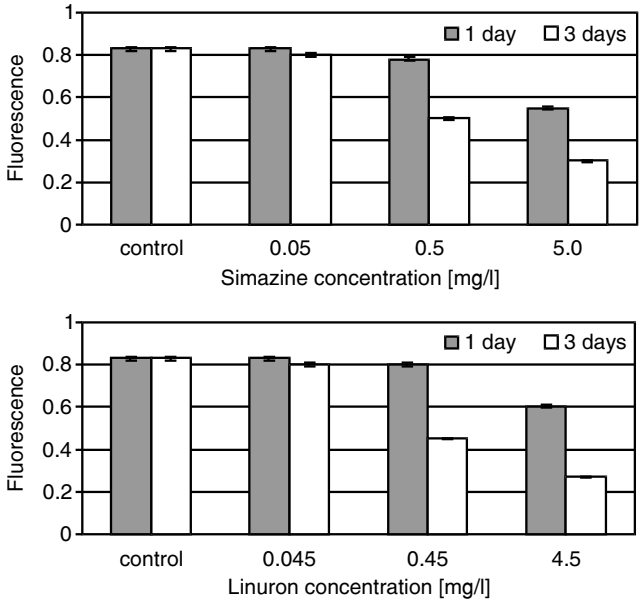

Fig. 2. Potential efficiency of PS2 (Fv/Fm). The variable fluorescence parameter Fv/Fm was determined after a 20 min of dark adaptation for all samples of leaves. The ratio of Fv/Fm independently of time exposition to herbicide was reduced similarly compare with control.

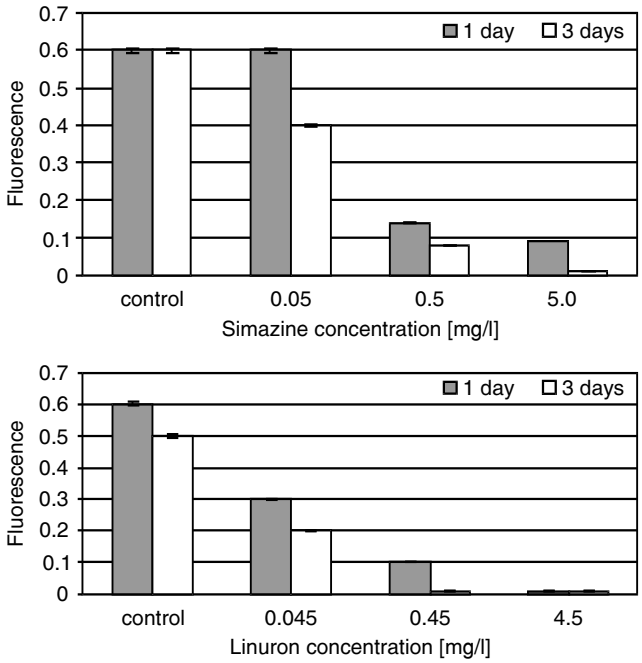

Fig. 3. Actual PS2 quantum efficiency (ΦPS2). Over the same range of simazine and linuron concentration the actual PS2 quantum efficiency (ΦPS2) after 3 days exposition was more inhibited at linuron (90%, 100% and 100% respectively) to compare 50%, 90%, 100% respectively at simazine.

Figure 5 shows the results of the assays of dry matter of cucumber seedlings cultivated for 3 days in the presence of both herbicides. No significant differences were observed except for the highest concentration of herbicides. Linuron only slightly lowered dry matter of seedlings in comparison with simazine.

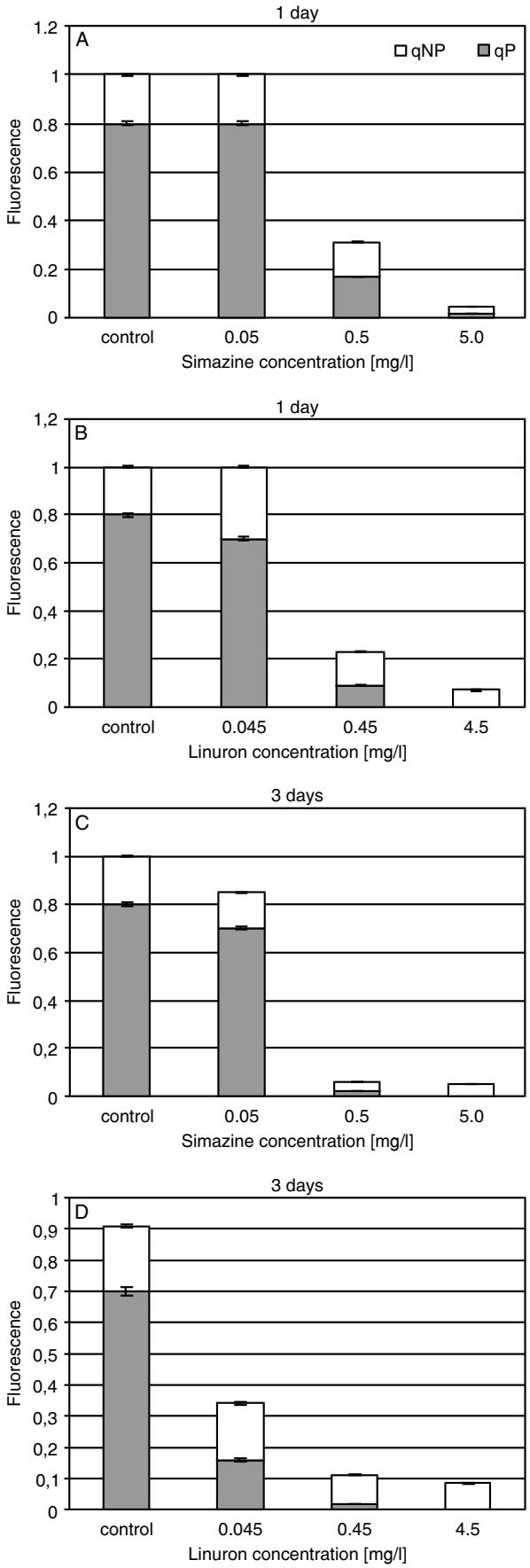

Fig. 4. Phemiotochcal and non-photochemical quenching of chlorophyll fluorescence. Phemiotochcal and non-photochemical quenching of chlorophyll fluorescence were measured on leaves of cucumber seedlings after herbicide treatment. The concentration of simazine and linuron was the same as described earlier.

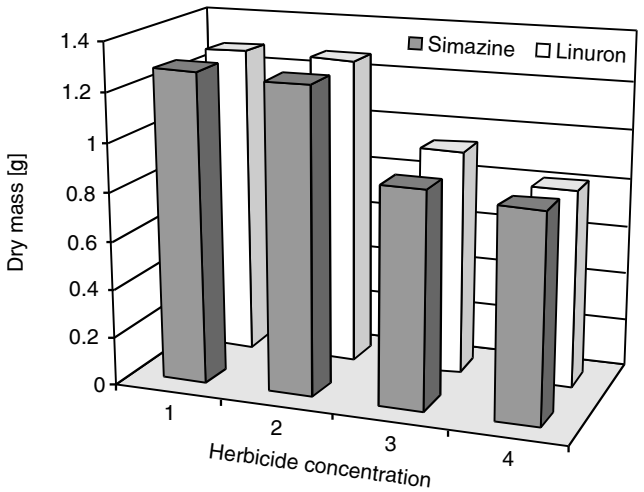

Fig. 5. Dry mass of cucumber seedlings. Cucumber seedlings grew during 3 day on nutrient solution without herbicides (control) or on solution with simazine and linuron After 3 days the seedlings were harvested and dried at 60˚C during 4 days, then dry mass every combination was occurred.

DISCUSSION

Inhibitory effect of herbicides on weed growth has been reported by many authors (Ashton and Crafts 1981; Vecchia et al 2001; Praczyk and Skrzypczak 2004; Nemat et al. 2005). In the present study photosynthetic activity of cucumber seedlings was reduced in response to simazine and linuron treatment. The translocation of both of them from roots to leaves is fast and after 24 hours we observed a decrease of net photosynthetic rate (PN) (Fig. 1). Reduction of PN in cucumber leaves after herbicide treatment was probably not due to the direct effect of herbicide on the stomata closure, because GS values (data not shown) were not changed in the treated leaves in comparisson with the control leaves. A drop in CO_2 reduction rate during photosynthesis confirms the hypothesis that dark metabolism of carbon is depending on the flow of electrons in the light phase of photosynthesis (lower Fv/Fm and ΦPS2), (Hopkins and Hüner 2004).

A strong effect of both of herbicides was observed on the potential PS II efficiency (0.550-0.600 for both after 1 day compared to 0.830 for the control; and about 0.300 after 3 days compared to 0.830 for control) (Fig. 2). According to Björkman and Demming (1987) or Frachbout and Leipner (2003) if Fv/Fm is the same or higher than 0.8, the potential efficiency of photosystem II is not affected. The disturbances in this efficiency (Fv/Fm) under herbicides stress depend on time of exposure to these herbicides and their concentration in nutrient solution, as well as under other stresses (Allakhverdiev et al. 2000).

Inclusion of linuron to nutrient solution decreased markedly the actual quantum efficiency (ΦPS2) in leaves of seedlings as compared with leaves of seedlings treated with simazine (Fig. 3). The difference in the sensitivity of cucumber leaves to linuron treatment might be due to the specific binding of urea-type herbicides to the acceptor side or the reducing side of PS II (Hsu et al. 1986). On the other hand, reduction of ΦPS2 could be explained by the decreased capacity of the carbon metabolism and lower utilization of NADPH and ATP in dark phase of photosynthesis (Subrahmanyam and Rathore 2000).

Light absorption results in excitation of chlorophyll molecules, which can return to the ground state by one of several pathways. Excitation energy (as Chl fluorescence) can be transferred to reaction centers of PS II and used to drive photochemistry (qP) or can be de-excited by energy dissipation as heat (qNP). Plants are able to maintain a low steady-state fluorescence yield due to combination of qP and qNP (Müller et al 2001; Niygoi et al 2004). Both the herbicides at the highest concentration strongly decreased photochemical quenching of fluorescence and non-photochemical quenching (Fig. 4A-D).

At lower concentration of both herbicides only a mild effect on dry matter of seedlings was observed (Fig. 5). Linuron as well as simazine decreased DM of seedlings at their highest concentrations.

CONCLUSIONS

The results of the present study showed that exposure of cucumber seedlings to simazine or linuron strongly decreased the photosynthetic electron transport. On the other hand, influence of both herbicides on the dark photosynthetic reactions is rather indirect but also strong. It is noteworthy that 3 days after the herbicides application all of photosynthetic parameters were significantly lower in comparison to the 1st day but decrease of seedlings growth after 3 days was about 40% only as compared with control.

Both herbicides kill plants by inhibiting photosynthesis but triazines are the most widely used agricultural pesticides in the world (especially on fields of corn). Simazine has a half-life in soil from one week to one year, depending on soil conditions. Because of an ongoing large-scale use of triazine herbicides, simazine can be found at high levels in the environment for example in drinking water. On the other hand, weeds that are heavily treated with triazine herbicides can become resistant (Hirschberg and McIntosh 1983; Sato et al. 1988; Alfonso et al. 1996). Urea-type herbicides are better soluble in water and have a shorter half-life in soil than triazines. Not many weeds are resistant to linuron (Newman and Adam 2003). Both the herbicides are categorized as possible human carcinogens. Urea-type herbicides are not detected as frequently as triazine-type ones in the environment, and they have great strength of killing weeds, because of this reason they may be replaced by triazines in agriculture (Newman and Adam 2003).

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