Can the *Giberella zeae* toxin zearalenone affect the photosynthetic productivity and increase yield formation in spring wheat and soybean plants?

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Abstract

The seeds of soybean *cv.* Aldana and spring wheat *cv.* Torka were soaked for 24 h in solution of zearalenone [ZEN, 2,4-dihydroxy-6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-benzonic acid lactone, 4 mg dm^{-3}] and then they were sown in the pot experiment in an open vegetation hall. The after-effects of ZEN on growth of plants, net photosynthetic (P_N) and transpiration (E) rates, stomatal conductance (g_s), photochemical efficiency of photosystem II (PSII) and on final seeds yield, were determined. A significant increase of seeds yield was revealed in plants of both cultivars *i.e.* by 22% and 19% of seed (grain) number and by 28 and 24% of seed (grain) mass, in soybean and in wheat, respectively. The photosynthetic rate (P_N) was stimulated during the juvenile and final phase by about 13.6% (average) in soybean plants. During other developmental stages, assimilation of CO_2 was retarded. The response of CO_2 assimilation in wheat plants was less pronounced as compared to that in soybean, but an increase of P_N by over 24% near the final stage of development was observed. The quantum yield of PSII electron transport (Φ_{PSII}) in soybean plants was changed after the treatment of seeds by ZEN similarly as for the rate of CO_2, whereas in wheat it continued to gradually increase *i.e.* during the whole growth period. Changes of Φ_{PSII} both in soybean and in wheat plants, as the response to ZEN treatment, were accompanied with an increase in the efficiency of changes occurring within the antenna (F_v'/F_m') as well as within centres of photochemical reactions (q_p). The conclusion is that ZEN can affect plant growth and development in many ways, as well as in the status and functioning of the photosynthetical apparatus. Some of the effects can be very long-lasting, as *e.g.* stimulation of production of seed yield in response to treatment of seeds with this substance.

Additional key words: chlorophyll fluorescence; gas exchange; growth analysis; productivity; soybean; wheat; zearalenone.

Introduction

Zearalenone [ZEN, 2,4-dihydroxy-6-(10-hydroxy- 6-oxo-*trans*-1-undecenyl)-benzonic acid lactone], an estrogenic substance (Christensen *et al*. 1965), is a secondary metabolite produced by *Gibberella zeae* (Stob *et al*. 1962). It acts as sex-regulating hormone in certain fungi (Wolf and Mirocha 1973). ZEN is one of the frequently occurring contaminants in cereals and plant products (Zinedine *et al*. 2007). It exhibits estrogenic effects and presents a problem for agriculture mainly by causing diseases in the reproductive system, impaired fertility, and abnormal fatal development in farm animals. Some studies have indicated that exogenous ZEN influenced plant growth and development. Meng *et al*. (1992) also found that ZEN was an endogenous regulator controlling plant development. A peak of endogenous ZEN levels occurred during the vernalization of many winter plants, and exogenous

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Abbreviations: DAS – days after sprouting; E – transpiration rate; F_v'/F_m' – efficiency of excitation energy capture by open PSII reaction centres; g_s – stomatal conductance; LAR – leaf area ratio; MTS – mass of thousand seeds; NAR – net assimilation rate; NPQ – nonphotochemical quenching; PFD – photon flux density; P_N – net photosynthetic rate; PSII – photosystem II; q_P – photochemical quenching coefficient; RGR_A – relative growth rate of the leaf area; RGR_W – relative growth rate of the plant biomass; WUE – water use efficiency; ZEN – 2,4-dihydroxy-6-(10-hydroxy- 6-oxo-*trans*-1-undecenyl)-benzonic acid lactone; Φ_{PSII} – quantum yield of PSII electron transport.

ZEN can partly replace the low-temperature requirement for ear development in winter wheat (Fu and Meng 1994; Fu *et al*. 2000). In combination with greatly shortened vernalization (2 weeks at 5°C), ZEN definitely eliminated the flowering blockade of winter wheat *cv*. Grana, which usually requires vernalization for 8–9 weeks (Biesaga-Kościelniak 2001). Moreover, ZEN changes the content of metabolites, such as fatty acids, phytosteroles, and modifies the course of the process of ion uptake by the plants (Biesaga-Kościelniak 2001). ZEN influences the intensity of growth of the whole plant including tissues and individual cells, the germination of caryopses, and even enables the induction of haploid embryos of wheat, as well as controlling the intensity of respiration and assimilation of CO_2 by the plants (Biesaga-Kościelniak *et al*. 2003, Szechyńska-Hebda *et al*. 2007, Biesaga-Kościelniak and Filek 2009). ZEN also affects such discrete physical parameters of the tissues as the electric surface potential as well as the permeability of the cell membranes to electrolytes (Biesaga-Kościelniak 2001). Some studies indicate that ZEN inhibits cell membrane transport of maize roots (Vianello and Macri 1978) and enhances α-amylase and β-glucosidase activities of germinating maize seeds (Vianello and Macri 1982).

The objective of our work was to answer the questions, if- and how ZEN introduced into seeds before germination can influence processes that are significant for seed yield production in plants. As Filek *et al.* (2007) showed that ZEN can affect the reconstruction of chloroplast membranes in rape plants, the effects of ZEN on the processes of growth and biomass accumulation as well as on CO_2 assimilation and photochemical efficiency of PSII in plants were included in our experiment. Soybean and wheat plants were selected as they have the distinct requirements for assimilates because, among others, of different energetic load of their seeds (Loomis 1983).

Materials and methods

Plants: The experiment was carried out on Polish common wheat (*Tritium aestivum* L.) *cv*. Torka, and on soybean (*Glycine max* (L.) Merrill) *cv*. Aldana. The wheat *cv*. Torka increases the grain yield under strong N nutrition (180 kg N ha^{-1}) and under intense plant protection to over 6 t ha^{-1} (Rachoń *et al*. 2002a). This cultivar is resistant to logging, and the mass of 1000 grains can amount to 45–52 g (Rachoń *et al*. 2002b). Soybean *cv*. Aldana is coarse-grained and its seeds yield in good weather conditions can exceed 2 t ha^{-1}, and the mass of 1000 seeds can amount to about 240 g, with the content of oil and protein being 40% and 15%, respectively (Michałek and Borowski, 2006).

Incubation of seeds with ZEN and conditions of plant growth: Seeds were soaked for 24 h in the solution of ZEN (*Sigma*, Poznań, Poland) at a concentration of 4 mg dm^{-3} and separately in water (control). The methods of incubation and concentration of ZEN were chosen on the basis of previous experiments (Biesaga-Kościelniak *et al*. 2006a,b). The soaked seeds were sown into the soil-substratum in the pots, volume 5 l, with 3 plants per pot. Pots were kept in the nearly natural conditions of the open vegetation hall of the University in Krakow in 2006. The sprouting of wheat plants was observed on April 23 and that of soybean on May 21. The plants were watered every day with water and two times a week with Hoagland's nutrient (Hoagland and Arnon 1938). The water content in the soil-substratum was controlled using sensor *(Hydrosense^{TM} Campbell Scientific*, *Inc*. Logan, Utah, USA). Air temperature was monitored every 5 min during the whole experiment using sensors located near the top of plants.The after-effect of seed soaking in ZEN solution on the rate of CO_2 assimilation was also determined in a separate field experiment, in the fully expanded mature upper leaves of soybean plants (*cv*. Aldana) and of spring wheat (*cv*. Torka). The measurements were carried out at the time between 10 and 14 h during sunny days on June 12 and on June 28, 2007.

Growth analyses of wheat plants were made on: May 3 (2-leaves stage), May 15 (3 leaves, tillering), May 26 (4 leaves) and June 12 (young ears visible), while those for soybean were conducted on: June 22 (stage of 2 leaves), July 5 (4 leaves), July 19 (8 leaves, young pods visible), August 2 (rapid pods growth). Leaf area was measured using a scanner (*ScanMaker 3880, Microtek, Hsinchu*, Taiwan) and *Delta-T Skan 2.03* software (*Delta-T Devices, Cambridge*, UK). Plant material was dried at 70°C and the following plant growth indexes were calculated according to Květ *et al*. 1971:

net assimilation rate:

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \frac{A_2^{\alpha-1} - A_1^{\alpha-1}}{A_2^{\alpha} - A_1^{\alpha}} \frac{\alpha}{\alpha - 1}$$

$$\alpha = \frac{RGR_W}{RGR_A}$$

relative growth rates of the plant biomass and leaf area:

$$RGR_W = \frac{ln\, W_2 - ln\, W_1}{t_2 - t_1}$$

$$RGR_A = \frac{ln\, A_2 - ln\, A_1}{t_2 - t_1}$$

leaf area ratio:

$$LAR = \frac{A}{W}$$

where W_1 and W_2 is the plant dry mass at times t_1 and t_2, respectively, A_1 and A_2 is the leaf area at times t_1 and t_2, respectively.

Analysis of seed yield structure, concerning number of ears or pods, seed/grain number and mass, as well as mass of 1000 seeds/grains, were made on July 31 (wheat) and August 21 (soybean).

Gas exchange analysis: P_N, E and g_s were measured using an infrared gas analyzer (*Ciras-1*, *Hansatech*, *PP-Systems,* Hitchin, Herts, UK) with a Parkinson leaf chamber (*PLC6*) automatically controlling the measurement conditions. The irradiation system was equipped with halogen lamps. The flow rate of air with a constant CO_2 concentration [400 $cm^3(CO_2)$ m^{-3}(air)] through the assimilation chamber was equal to 350–400 cm^3 min^{-1}. The measurements were made on the middle part of intact leaf (upper side) at 25°C (the leaf temperature), irradiance was equal to 1200 µmol(quantum) m^{-2} s^{-1} and relative humidity (RH) equal to 30%. The adaptation of leaves to irradiation was continued until maximum and stable gas exchange rate was observed.

Measurements of the photochemical efficiency of PSII: A modulated fluorescence system *FMS2* (*Hansatech Ltd.,* Kings Lynn, UK) was used. The measurements were made on the middle part of the leaves at 25°C. The source of the modulation beam (duration pulses 1.8 µs, 2.3 kHz) was the amber LED [peak wavelength 594 nm, PFD *ca*. 0.05 µmol(quantum) m^{-2} s^{-1}]. Actinic [white light; 400–750 nm; 500 µmol(quantum) m^{-2} s^{-1}] and pulse irradiations were provided by a halogen lamp (*Osram 64255*; 20 W). Upper side of intact leaf was illuminated for a period longer than 5 min until stabilization of fluorescence (F_s). The saturating pulse (F_m') had an intensity of about 5800 µmol(quanta) m^{-2} s^{-1} and lasted 0.9 s. F_0' was measured after turning off the actinic light, by immediately irradiating the leaf for 3 s with a far-red emitting diode (wavelength 735 nm) with about 15 W m^{-2}. The efficiency of excitation energy capture by open PSII reaction centres (F_v'/F_m'), the photochemical quenching (q_p), the quantum yield of PSII electron transport (Φ_{PSII}), and the nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence were determined (Genty *et al*. 1989; Lichtenthaler *et al*. 2005). One part of the fluorescence measurements were taken on dark-adapted leaves (30 min in leaf clip).

Statistical analysis: All data were analyzed using *Statistica 8.0* software (*Statsoft Inc*., Tulsa, OK, USA). The experiment was a complete random design consisting of two ZEN treatments (0 and 4 mg ZEN dm^{-3}). The significance of differences of mean values taken from data with normal arrangement (*Shapiro-Wilk* test) was tested using the Student´s *t*-test. For data that did not respond to that criterion, the *Mann-Whitney U* was applied.

Results

Plant growth and seed yield production: High temperature during vegetation (Fig. 1) and a good water supply as well as mineral fertilization allowed the rapid growth of plants. In soybean plants that were grown from seeds incubated in ZEN solution, a stimulation of increase in leaf area and the mass of leaves and stems during the major part of the developmental period were observed (Table 1). However, during the early stage: 32–59 days after sprouting (DAS), the relative growth rate of plant mass (RGR_W) and that of the leaf area (RGR_A) as well as NAR, were retarded when compared to control (–ZEN). The significant increases of NAR and RGR_W by 40% and 35%, respectively, and several times of RGR_A were observed during the period between 59 and 73 DAS. Finally the number of seeds per plant increased by over 22%, their mass by over 28% and the seed mass per pod - by 20%. The increase of seed yield was not accompanied with decreases in the mass of 1000 seeds.

The response of wheat grown on ZEN treatment was less pronounced than that of soybean. During the early stage of development (10 DAS) the growth of leaves and stalk were strongly retarded. During further stages, the temporal RGR_W increase RGR_A were observed, and finally the grain yield per plant and per ear was increased by 25% and 14%, respectively. It was a result of the grain number increase by over 19% but not an increase in the mass of 1000 seeds.

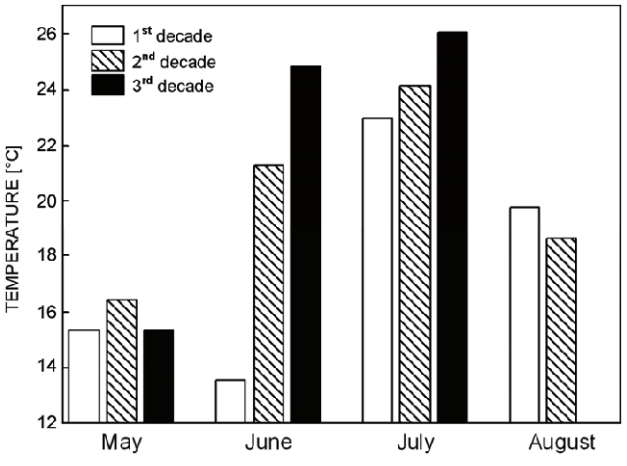

Fig. 1. Air temperature during growth of wheat and soybean in the open vegetation hall.

Table 1. The after-effect of pre-sowing soaking of soybean and spring wheat in zearalenone (ZEN) solutions on the plants growth and on yield structure. NAR – net assimilation rate [g(shoot) m^{-2}(leaves) d^{-1}]; DAS – days after sprouting; DM – dry mass; LAR – leaf area ratio [m^2(leaves) g^{-1}(shoot)] × 10^3; MTS – mass of thousand seeds [g]; RGR_A – relative growth rate of the leaf area [m^2(leaves) m^{-2}(leaves) d^{-1}]; RGR_W – relative growth rate of the plant biomass [g(shoot) g^{-1}(shoot) d^{-1}] × 10^2. [a)] seeds soaked 24 hrs in water (–ZEN) and in a zearalenone solution (4 mg dm^{-3}, +ZEN). [b)] changes of values in percent of control (–ZEN), * significant differences ($P<0.05$) according to the Student´s *t*-test (parameters 1–4, 9, 11–13) and of the *Mann-Whitney U* test (parameters 5–8, 10), means of 25–37 repetitions (***bold numbers***).

Soybean

DAS	Method of incubation[a)]	Leaf area [cm^2]	DM [mg]			LAR	RGR_A	RGR_W	A_N	Number of seeds per plant	Number of fertile pods	Seed DM per:		MTS
			leaves	stem	shoot							plant	pod	
		1	2	3	4	5	6	7	8	9	10	11	12	13
32	–ZEN	74.3	209.8	187.5	397.3	18.7								
	+ZEN	96.1	278.8	218.0	496.8	19.3								
	%% [b)]	**+29.3***	**+32.9***	**+16.3***	**+25.0***	+3.4								
45	–ZEN	157.9	669.4	531.3	1200.7	13.2	5.80	8.51	5.57					
	+ZEN	175.5	702.9	560.9	1263.8	13.9	4.63	7.18	4.48					
	%%	**+11.1***	**+5.0***	**+5.6***	**+5.3***	+5.6	**–20.1***	**–15.6***	**–19.7***					
59	–ZEN	391.7	1883.5	1372.4	3847.0	10.2	6.49	8.32	7.35					
	+ZEN	364.1	1753.9	1317.5	3545.6	10.3	5.21	7.37	6.31					
	%%	**–7.0***	**–6.9***	–4.0	**–7.8***	+0.9	**–19.7***	**–11.4***	**–14.1***					
73	–ZEN	416.5	2277.9	1975.7	7443.7	5.6	0.47	5.08	6.85					
	+ZEN	454.9	2688.8	2397.8	8615.5	5.3	1.71	6.83	9.56					
	%%	**+9.2***	**+18.0***	**+21.4***	**+15.7***	–5.6	**+262.7***	**+34.5***	**+39.6***					
92	–ZEN									25.2	18.5	5.64	0.30	223.8
	+ZEN									30.8	20.1	7.24	0.36	235.1
	%%									**+22.2***	+8.6	**+28.4***	**+20.0***	+5.0

Table 1 (continued)

Wheat

DAS	Method of incubation	Leaf area	DM			LAR	RGR_A	RGR_W	A_N	Number of grain per plant	Number of fertiled ears	Grain DM [g]:		MTS
			leaves	stalk	shoot							plant	ear	
		1	2	3	4	5	6	7	8	9	10	11	12	13
10	–ZEN	15.6	36.3	10.0	46.3	33.7								
	+ZEN	15.6	29.4	8.3	37.7	41.4								
	%%	0.0	**–19.0***	**–17.0***	**–18.6***	**+22.8***								
22	–ZEN	72.9	201.9	74.8	276.7	26.3	12.85	14.90	5.17					
	+ZEN	78.9	203.4	74.8	278.2	28.4	13.51	16.66	5.13					
	%%	+8.2	+0.7	0.0	+0.5	**+7.6***	+5.1	**+11.8***	–0.7					
33	–ZEN	168.9	530.8	244.5	775.3	21.8	7.64	9.37	3.97					
	+ZEN	161.9	522.5	269.2	791.7	20.4	6.53	9.51	4.04					
	%%	–4.1	–1.6	+10.1	+2.1	–6.1	**–14.5***	+1.5	+1.9					
50	–ZEN	244.4	842.8	448.7	2107.8	11.6	2.17	5.88	3.84					
	+ZEN	245.8	847.6	446.9	2125.8	11.6	2.46	5.81	3.91					
	%%	+0.6	+0.6	–0.4	+0.9	–0.3	+13.0	–1.2	+1.8					
99	–ZEN									113.1	4.2	5.27	1.25	46.6
	+ZEN									134.7	4.6	6.56	1.43	48.7
	%%									**+19.1***	+9.5	**+24.5***	**+14.4***	+4.5

Table 2. The after-effect of pre-sowing soaking of soybean seeds and wheat grains in ZEN solution on the gas exchange of plants. The measurements were carried out on the flag leaf and fully expanded mature upper leaf of soybean. P_N – net photosynthetic rate [μmol(CO_2) $m^{-2}s^{-1}$]; E – transpiration rate [mmol(H_2O) $m^{-2}s^{-1}$]; g_s – [mmol(H_2O) m^{-2} s^{-1}]; WUE – water use efficiency [μmol (CO_2) $mmol^{-1}(H_2O)$]. [a]seeds soaked 24 h in water (–ZEN) or in a zearalenone solution (4 mg dm^{-3}, +ZEN). [b]changes of values in percent of control (–ZEN), * significant differences (P<0.05) according to Student´s t-test, means of 9–11 repetitions (*bold numbers*).

DAS	Method of incubation[a]	P_N	E	g_s	WUE
Soybean					
33	–ZEN	17.7	4.7	222.2	3.77
	+ZEN	20.4	6.5	380.7	3.14
	%% [b]	**+15.3***	**+38.3***	**+71.3***	**–16.7***
45	–ZEN	21.2	8.2	324.8	2.59
	+ZEN	18.3	6.4	287.9	2.86
	%%	**–13.7***	**–22.0***	**–11.4***	**+10.6***
59	–ZEN	20.5	5.0	238.3	4.10
	+ZEN	18.1	4.1	169.3	4.41
	%%	**–11.7***	**–18.0***	**–29.0***	**+7.7***
73	–ZEN	19.4	6.1	247.7	3.18
	+ZEN	21.7	6.3	285.1	3.44
	%%	**+11.9***	+3.3	**+15.1***	**+8.3***
Wheat					
10	–ZEN	20.9	6.5	290.2	3.22
	+ZEN	21.9	7.0	318.0	3.13
	%%	+4.8	**+7.7***	+9.6	–2.7
22	–ZEN	21.8	5.2	232.8	4.19
	+ZEN	22.4	5.6	256.9	4.00
	%%	+2.8	**+7.7***	**+10.4***	–4.6
33	–ZEN	19.0	6.4	194.4	2.97
	+ZEN	19.7	6.9	188.8	2.86
	%%	+3.7	**+7.8***	–2.9	–3.8
50	–ZEN	16.8	3.6	146.8	4.67
	+ZEN	20.9	4.6	199.0	4.54
	%%	**+24.4***	**+27.8***	**+35.6***	–2.6

CO_2 assimilation and photochemical PSII efficiency: The CO_2 assimilation of soybean plants that were grown from seeds soaked in ZEN was stimulated at the beginning (33 DAS) and near the end of the plants development (73 DAS); its average value amounted to over 13% (Table 2). During the other periods (45 and 59 DAS), the photosynthetic rate was decreased by 12–14%. The changes of CO_2 assimilation were parallel to that of stomatal conductivity. The stimulation of P_N in soybean plants at the early stage of development was accompanied with a very strong increase in transpiration (over 38%) and this was the reason that the value of WUE then reduced for 16.7%. In the other stages, the values of WUE were increased by about 7–11%.

The response of wheat plants to the ZEN treatment was weaker than that of soybean ones (Table 2); only during the last measurement (50 DAS) there was a significant (over 24%) increase of P_N shown. During the whole growth season a significant increase of transpiration was observed and this effect was the most visible on 50 DAS (about 28%) and it could be the reason for a strong increase of g_s. On the contrary to soybean plants, there were no observed changes of E/P_N values in the wheat ones.

The high values of F_v/F_m observed in the leaves of both soybean and wheat seem to show the good form of the plants (Table 3). The quantum yield of PSII electron transport (Φ_{PSII}) in soybean plants was changed as a result of ZEN treatment similarly as that of CO_2; the increase of its value by 22% and 26% was observed as well during early (33 DAS) and final (73 DAS) measurements, respectively. In young soybean plants (33 DAS), this effect was accompanied by the increase of efficiency of excitation energy capture by open PSII reaction centres (F_v'/F_m' increase), whereas in older plants (73 DAS) it was connected rather with the increase in a photochemical suppression of fluorescence (q_p). The greater effectiveness of energy use for the transport of electrons in the PSII of older soybean plants which were grown from ZEN-treated seeds was accompanied with a decrease in the energy dissipation as heat in the PSII antenna complexes (decrease of NPQ by about 11%). In the other dates, the effect of ZEN was not visible (45 DAS) or in terms of decreasing the effectiveness of some photochemical reactions in PSII (59 DAS).

The measurements of fluorescence in wheat revealed that in plants grown from ZEN-treated seeds, the efficiency of photochemical reactions in PSII gradually increased over the whole vegetation period. At first (22 DAS), a small increase in Φ_{PSII} was revealed, later (33 DAS) an increase in F_v'/F_m' and Φ_{PSII}, followed on 50 DAS by a strong increase of F_v'/F_m', q_p and Φ_{PSII} values.

Discussion

The presented results evidently show that the incubation of seeds in the solution of ZEN can cause long-lasting after-effects, enabling a significant increase in grain yield production in wheat and soybean plants. Although the observed modulation of some processes in plants as a result of previous treatment of seeds with ZEN does not clearly explain the mechanism by which it works, it seems that stimulation of seed yield production is the result of both increase of grain number per plant and good grain filling. An enhanced mass of 1000 grains of

Table 3. The after-effect of pre-sowing soaking of soybean seeds and wheat grains in a ZEN solution on the photochemical activity of PSII in leaves. The measurements were carried out on the flag leaf and the fully expanded mature upper leaf of soybean. [a)]seeds soaked 24 h in water (–ZEN) or in a zearalenone solution (4 mg dm^{-3}, +ZEN). [b)]changes of values in percent of control (–ZEN), * significant differences ($P<0.05$) according to the Student's *t*-test, means of 5–7 repetitions (*bold numbers*).

DAS	Method of incubation[a)]	F_v/F_m	F_v'/F_m'	q_p	Φ_{PSII}	NPQ
Soybean						
33	–ZEN	0.817	0.389	0.303	0.118	2.893
	+ZEN	0.813	0.458	0.314	0.144	2.848
	%% [b)]	–0.5	**+17.7***	3.6	**+22.0***	–1.6
45	–ZEN	0.815	0.445	0.218	0.097	2.891
	+ZEN	0.820	0.448	0.217	0.097	2.742
	%%	+0.6	+0.7	–0.5	0.0	–5.2
59	–ZEN	0.837	0.522	0.454	0.237	2.541
	+ZEN	0.838	0.517	0.401	0.207	2.593
	%%	+0.1	–1.0	**–11.7***	**–12.7***	+2.0
73	–ZEN	0.804	0.504	0.215	0.108	2.050
	+ZEN	0.809	0.544	0.250	0.136	1.828
	%%	+0.6	+7.9	**+16.3***	**+25.9***	**–10.8***
Wheat						
10	–ZEN	0.814	0.434	0.361	0.157	3.512
	+ZEN	0.809	0.411	0.380	0.156	3.681
	%%	–0.6	–5.3	+5.3	–0.6	+4.8
22	–ZEN	0.832	0.421	0.442	0.186	3.654
	+ZEN	0.822	0.433	0.455	0.197	3.546
	%%	–1.2	+2.9	+2.9	**+5.9***	–3.0
33	–ZEN	0.823	0.397	0.444	0.176	3.703
	+ZEN	0.822	0.424	0.465	0.194	3.455
	%%	–0.1	**+6.8***	+4.7	**+10.2***	**–6.7***
50	–ZEN	0.821	0.430	0.437	0.188	3.331
	+ZEN	0.825	0.465	0.487	0.226	3.147
	%%	+0.5	**+8.1***	**+11.4***	**+20.2***	–5.5

soybean and wheat seems to be connected with an increase of the NAR and/or CO_2 assimilation rate as well as with the stimulation of the photochemical efficiency of PSII, during the final phase of plant development and also with more effective translocation of matter from vegetative parts into seeds. It is worth emphasising that the increase of heading in wheat and pod setting in soybean as well as the increase of grain number as a response to ZEN treatment had already been observed (Biesaga-Kościelniak *et al*. 1998, Biesaga-Kościelniak *et al*. 2006a,b). It was shown that the effectiveness of ZEN depended on its concentration, application method and on variety. The after-effects of ZEN treatment appeared as well when its solution was used for seed soaking, plants watering or for spraying them.

The stimulating effect of treatment of seeds with ZEN on the CO_2 assimilation rate in plants of the same cultivars of soybean and wheat was confirmed in an experiment carried out under natural conditions in the field (Fig. 2). These results are also consistent with the earlier observations (Biesaga-Kościelniak 2001) that ZEN used in a wide range of concentration stimulated photosynthesis in maize leaves in hydroponics.

As in our experiment, the after-effect of the seeds treatment with ZEN was the increase in photochemical activity of PSII, which was observed at the early and at the final developmental stages in soybean, when the increase in the CO_2 assimilation rate took place. Also, it seems possible that the increase of CO_2 assimilation was the result of increasing the efficiency of energy flow through PSII. As concerns the wheat plants, measurements of fluorescence showed a progressive increase of PSII stimulation during their developmental period in response to seed treatment with ZEN. This effect occurred for a long time without a simultaneous increase of CO_2 assimilation, but it changed at the end of the vegetation period after a strong stimulation of quanta efficiency of electron transport in PSII. The results of fluorescence measurements allow us to ascertain that the increase and retardation of Φ_{PSII} after ZEN treatment can be caused within the antenna system (F_v'/F_m' changes) as well as within photochemical centres (q_p changes).

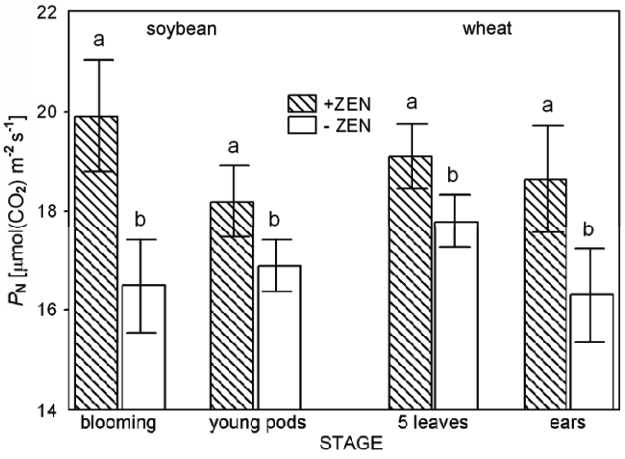

Fig. 2. The after-effect of seed soaking in ZEN solution on the rate of CO_2 assimilation in fully expanded mature upper leaves of soybean plants (*cv*. Aldana) and of spring wheat (*cv*. Torka). The measurements were carried out between 10 and 14 h on sunny days in 2007 on June 12 and on June 28. Mean temperature of leaves amounted to 27–31°C. The seeds were soaked in ZEN solution (4 mg dm^{-3}, +ZEN) and in water (–ZEN) for 24 h. Different letters over bars mean significant differences according to the Student´s *t*-test (P<0.05), means of 12–18 repetitions ± SD.

Our results show that the after-effects of seed treatment with ZEN are varied depending on a plant development stage; may be it has a stimulating effect in one phase and a retarding effect in another one. The rather scarce results of some authors (Mayr 1988, Filek *et al*. 2002, Gzyl *et al*. 2004, Filek *et al*. 2007) do not allow us to explain these changes but only make some suppositions concerning the mechanism of ZEN action in plants. One of the suggestions is that ZEN can cause a strong alteration of metabolism through activating the estrogen receptor in plant tissues. Stimulation of the activity of the photosynthetic process by ZEN probably does not occur in all species. In the experiments with gram (*Cicer arietinum* L.) and mustard (*Brassica juncea* L.) it was discovered that seed incubation caused after-effects such as the strong inhibition of the synthesis of chlorophylls *a*, *b* and carotenoids (Kumar and Sinha 1995). The response as the above one was explained by the disturbance of the pigment synthesis by restricting the growth-hormone-induced synthesis of RNA, DNA and proteins in the leaf. As ZEN changes the distribution of cell membrane charges, and it can affect the reconstruction of chloroplast membranes in plants (Filek *et al*. 2007), a general opinion may be formulated that its action seems to be significant for chloroplast (and PSII) functioning, but further research is needed in this respect.

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