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A long-term response of chlorophyll fluorescence induction to one-shot application of cyanazine on barley plants and its relation to crop yield

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Abstract

Field-grown plants of spring barley (*Hordeum vulgare* L. cv. Akcent) in the growth phase 30 DC (beginning of stem extension) were exposed to a one-shot application of a commercial product containing cyanazine (*Bladex 50 SC*) in two doses, C_{30} and C_{60} (30 and 60 mg m^{-2}). The reaction of the plant photosynthetic system was followed non-destructively using chlorophyll fluorescence induction (the O-J-I-P transient) within three weeks after the application in the fifth developed leaf and three further gradually appearing leaves. An immediate response of plants to the application of cyanazine and a regeneration of plants from cyanazine action were detected. The biological (plant dry mass) and crop yield production (the number and mass of grains in a spike) were analyzed in time of full ripeness. The crop yield was lowered by the herbicide effect to the same level for the two doses used.

Additional key words: *Bladex 50 SC*; caryopsis mass; grain number and mass in spike; herbicide; *Hordeum vulgare*; leaf age; plant dry mass; plant topography.

Introduction

Many herbicides inhibit photosynthesis (Fedtke 1982). Herbicides of urea, triazine, and phenolic types inhibit photosynthetic electron transport of photosystem 2 (PS2-herbicides) (Briantais *et al.* 1986). Their mode of inhibition of PS2 function consists mostly in a displacement of the plastoquinone molecule (Q_B) from its binding site on the D1 protein (Velthuys 1981, Wraight 1981). The binding of the PS2-herbicide to

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Abbreviations: CE, characteristic extreme; Chl, chlorophyll; CM, caryopsis mass; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; GN, grain number in spike; MG, mass of grains in a spike; PEA, Plant Efficiency Analyser; PS, photosystem; TG, topographic gradient.
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this niche prevents a reoxidation of the reduced primary quinone acceptor Q_A^- and blocks the electron flow (Velthuys 1981, Vermaas *et al.* 1984). There is an additional effect that might have even more important consequences than the blocking of the electron transport: the inhibition of the fast degradation and biosynthesis (the rapid turnover) of the herbicide binding D1 protein. The damaged D1 protein can be repaired only by its displacement from the PS2 complex and a complete resynthesis of the polypeptide and reassembly of PS2. These turnover and repair mechanisms are prevented by most of the PS2-herbicides (Mattoo *et al.* 1984, Komenda 1998). The interference of the herbicides with the D1 protein turnover would sustain and aggravate the inhibition of PS2 (Draber *et al.* 1991).

After the application of a herbicide solution into soil or on leaves, a heterogeneous distribution of the herbicide molecules occurs in the plant. To study it, the chlorophyll (Chl) fluorescence methods are often used at present (Papageorgiou 1975). They are rapid, non-destructive, and directly reflect the binding of the PS2 herbicide to the active site. They have been successfully used in investigations of herbicide perturbation in PS2 both *in vitro* and *in vivo* (*e.g.*, Brewer *et al.* 1979, Laskay and Lehoczki 1986, Ducruet *et al.* 1993, Joshi *et al.* 1994). At very high excitation irradiances, two steps (I_1, I_2) between the O and P phases of the fluorescence rise are discernible (Neubauer and Schreiber 1987, Schreiber and Neubauer 1987). At the introduction of the *PEA* fluorometer, an inflection was found at about 2 ms that was called „J" (Strasser and Govindjee 1992a,b). This J step is related to I_1 of Neubauer and Schreiber (1987) or I step observed earlier by Delosme (1967) (Strasser *et al.* 1995, Lazár 1999). Due to a consistence with most of the literature on Chl fluorescence induction, the use of the O-J-I-P nomenclature is usually recommended (Govindjee 1995, Lazár 1999). The J step is very sensitive to PS2 herbicide action (Strasser and Govindjee 1992a, Strasser *et al.* 1995, Srivastava *et al.* 1995, Lazár *et al.* 1997, 1998).

Due to heterogeneous distribution of a herbicide in the plant, measurement in several representative parts of the plant is necessary in studies of herbicide translocation and detoxification. Recently, methods of fluorescence imaging have been developed for the physiological measurements and early stress detection including the action of herbicides (Daley *et al.* 1989, Daley 1995, Lichtenthaler and Miehe 1997, Lichtenthaler *et al.* 1998). The fluorescence imaging techniques inform, in contrast to the point data measurements, on the whole leaf area. However, in fluorescence measurements on the whole plants, especially within a canopy, the comparable optical conditions of leaves (*e.g.*, the intensity of excitation) from various canopy levels cannot be expected (Sowinska *et al.* 1996). Since the fluorescence signal strongly depends on the excitation conditions (Krause and Weis 1991, Strasser *et al.* 1995) the results obtained from the fluorescence imaging measurements with whole plants might be inaccurate.

In this work we suggest a simple method of fluorescence topography that allows to determine a response of the whole plant to a herbicide in a prolonged time interval and in field conditions. This method was applied to field-grown barley plants treated with a commercial product containing cyanazine (*Bladex 50 SC*). Topographic points on a plant for measurements of the O-J-I-P fluorescence transient were chosen. By

means of the method of fluorescence topography an immediate reaction of plants to the foliar application of herbicide, regeneration of the plants from the herbicide action, and an effect of the herbicide treatment on later plant ontogeny was followed. Different extent of the fluorescence changes due to different cyanazine dose was reflected in changes of individual parameters of crop yield.

Materials and methods

Plants and herbicide treatment: Seeds of spring barley (*Hordeum vulgare* L. cv. Akcent) were sown in soil on March 13, 1997 and grown in a field near Kroměříž, the Czech Republic (49°17'N, 17°21'E; 235 m above sea level). A commercially available herbicide product *Bladex 50 SC* (*American Cyanamid Co.*, USA) with concentration of cyanazine 500 g l^{-1} was applied on May 14, 1997 by spraying the leaves using a hand sprayer (*Solo 425*, Germany), in amounts of 0.6 or 1.2 litre per ha diluted in 300 litre H_2O. Thus the doses of cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile) were 30 and 60 mg m^{-2}, respectively. The barley plants were in the time of application in the growth phase 30 DC according to Zadoks *et al.* (1974). The fifth developed leaf on plant was designated as L1 leaf. The next appearing younger leaves were denoted L2 to L4 (see Fig. 1). In the time of herbicide application, the L2 leaves were erected and partly unrolled, so that they could be affected by the herbicide spraying but less than the mature L1 leaves. Only the leaves of the main stalks were used for the measurements.

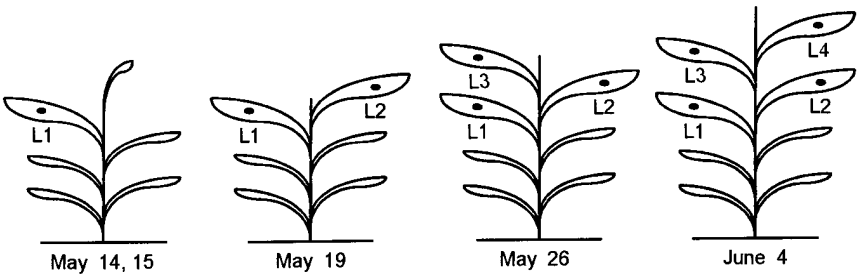

Fig. 1. Leaf numbering and leaf regions used for fluorescence measurement of gradually accruing leaves of field-grown barley plants. Measured in 1997. The herbicide cyanazine was applied on May 14, on plants in the growth phase 30 DC according to Zadoks *et al.* (1974).

Chl *a* fluorescence induction curves were measured with a portable Chl fluorometer *PEA* (Plant Efficiency Analyser, *Hansatech Instruments*, England), non-destructively on gradually appearing L1 to L4 leaves of barley plants within three weeks (see Fig. 1), always between 09:00 and 10:00 h. The measured leaf regions were dark-adapted in a leaf clip (*Hansatech*) for 30 min before the measurement. A fluorescence detection time of 2 s and an excitation intensity of about 3 000 μmol m^{-2} s^{-1} were used. Fluorescence parameters F_0, F_P, $F_V/F_P = (F_P - F_0)/F_P$ and $V_J = (F_J-F_0)/(F_P - F_0)$ were determined. F_J at the J step was evaluated from the experimental curves at time

of 2 ms. The V_J parameter (*e.g.*, Srivastava *et al.* 1995, Strasser *et al.* 1995) corresponds to the rF_J parameter used in Lazár *et al.* (1997), Lazár and Nauš (1998), or Bartošková *et al.* (1999). The F_V/F_P ratio correlates with the theoretical value F_V/F_M (Ögren 1988 and our unpublished results) representing the quantum efficiency of PS2 photochemistry (Krause and Weis 1991). The fluorescence parameters were statistically evaluated (medians and quartiles) as they may not obey the normal distribution (Lazár and Nauš 1998). In order to eliminate the effect of leaf ageing on Chl fluorescence parameters (Šesták and Šiffel 1997) the control plants were measured simultaneously with the treated ones.

Meteorological conditions during fluorescence measurements are presented in Table 1. Temperature and relative air humidity were measured by a thermohygrometer model *3309-60* (*Cole-Parmer*, USA) and PAR irradiance by a Quantum Radiometer *LI-189* (*Li-Cor*, USA).

Table 1. Mean temperature (*T*), relative air humidity (RH), and intensity of photosynthetically active radiation (PAR) in terms of chlorophyll fluorescence measurements on field-grown barley plants. Measured in 1997.

Date	*T* [°C]	RH [%]	PAR [μmol m^{-2} s^{-1}]
May 14	26.0	60	1255
15	23.5	66	1062
19	19.6	74	430
26	15.1	51	808
June 4	21.9	40	1523

Chl fluorescence topography (Matoušková *et al.* 1996, 1998): The central parts of the adaxial side of several consecutive leaf blades were chosen as the topographic points. Two quantities of the topography were determined—a characteristic extreme (CE) and a topographic gradient (TG). The characteristic extreme of a fluorescence parameter was defined as an extreme value within the group of measured values. Based on changes of the Chl fluorescence parameters during natural senescence of barley leaves (values not shown) and on behaviour of these parameters under the PS2 herbicide action (Strasser *et al.* 1995, Lazár *et al.* 1997), maxima for F_P and F_V/F_P and minima for F_0 and V_J were postulated as the characteristic extremes.

The topographic gradient TG of the fluorescence parameter has been defined as

$$TG\ (f_i) = 1 - (f_{min,i})/(f_{max,i})$$
$$TG\ (f_j) = (f_{max,j})/(f_{min,j}) - 1$$

where f_i stands for F_P and F_V/F_P, and f_j for F_0 and V_J, respectively. f_{min} and f_{max} indicate minimum and maximum measured values of the fluorescence parameter within the topographic points.

In control plants, the CE reflects that part of the plant which is in optimal functional state. In the treated plants, CE shows the part of plant which is least affected by the cyanazine action. The topographic gradient represents a gradient of the quantity within the plant and reflects the heterogeneity of the functional state of photosyn-

thetic apparatus. In general, a higher TG (*i.e.*, a higher heterogeneity) implies a worse state of the plant due to the natural senescence or a long-time stress effect (see Šesták 1985). The values measured on L1, L2, and L3 leaves on May 26 and June 4 were used for estimation of the parameters of fluorescence topography.

Growth and yield parameters of barley plants: In the growth phase of full ripeness (92 DC according to Zadoks *et al.* 1974) on July 26, 1997, the dry mass of the above-ground part of plants was estimated. Further, the number of spikes, the mass of grains in spikes of whole plant, in the spike of a main stalk and the other productive stalks, the number of grains in spike, and the mass of caryopses were determined.

Results

Chl *a* fluorescence: In L1 leaves (Fig. 2) in the first phase after the cyanazine application mainly an increase of the relative height of the J step occurred (Fig. 2, curve *b*) in comparison with a leaf of the control plant (Fig. 2, curve *a*). In the second phase, a pronounced increase of the fluorescence intensity in the O step (*i.e.*, F_0) was observed (Fig. 2, curve *c*).

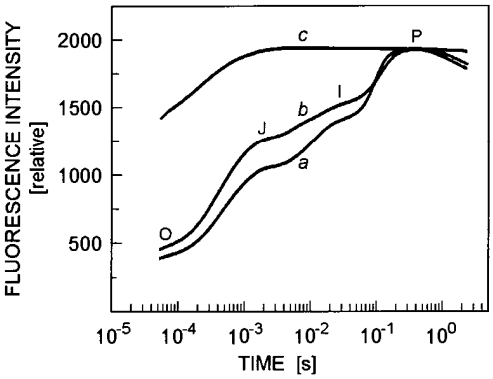

Fig. 2. The O-J-I-P fluorescence transient (measured with the *PEA* fluorometer, excitation irradiance 3000 μmol m^{-2} s^{-1}) in L1 leaves (the fifth developed leaves) of field-grown barley plants (growth phase 30-32 DC according to Zadoks *et al.* 1974). *a* - control; *b* and *c* - 1st and 5th d after the cyanazine application in a dose of 60 mg m^{-2}. Normalized on a fluorescence intensity in the P step of a control curve.

The lower dose of cyanazine (C_{30}) affected the V_J, F_0, and F_V/F_P Chl fluorescence parameters only in the L1 leaves directly exposed to the herbicide spraying (Fig. 3*A-C*). A significant increase of V_J by about 25 % was observed one day after the herbicide application. After 4 d, an additional increase of the V_J parameter occurred (by about 15 %) and even after 20 d V_J was by about 35 % higher than in the L1 leaves of the control plants. The real value (median) of the V_J maximum of the treated plants (5 d after application) was 0.73. The F_0 and F_V/F_P parameters of the L1 leaves responded to the herbicide treatment later than V_J (Fig. 3*B,C*). An increase of F_0 and a decrease of F_V/F_P were observed 5 d after the application. The F_0 increase in this

term of measurement was *ca.* 100 % of the L1 leaf of the control plants (Fig. 3*B*). In

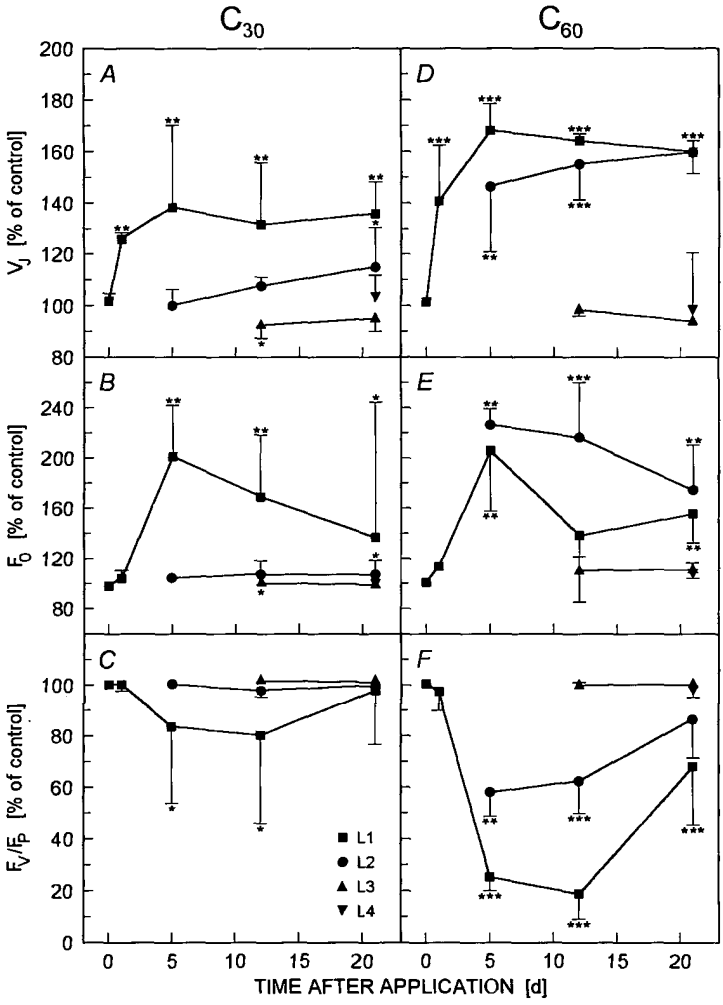

Fig. 3. Changes of fluorescence parameters V_J (*A, D*), F_0 (*B, E*), and F_V/F_P (*C, F*) in individual leaves of barley plants after the application of cyanazine in doses of 30 or 60 mg m^{-2} (C_{30} and C_{60}, respectively). L1 - the fifth developed leaf on plant, L4 - the eighth developed one. Medians and quartiles expressed in % of values of control plants are shown, $n = 10$. *,** significant differences at $p<0.05$ and $p<0.01$ compared to the control values, respectively.

the following measurement terms the F_0 parameter decreased, finally being about 30 % higher than that in the control plants. The slight decrease (by *ca.* 20 %) of F_V/F_P was observed 5 and 12 d after the application of cyanazine. In the last fluorescence measurement (*i.e.*, 21 d after herbicide application), F_V/F_P was similar in the treated and control plants. In younger leaves (L2-L4) which fully developed after application

of herbicide, only an increase of the V_J parameter in the L2 leaves was found. 12 and 21 d after application a very slight increase of this parameter occurred (Fig. 3*A*).

After the application of the double dose of cyanazine (C_{60}), changes of the fluorescence parameters were more pronounced (Fig. 3*D-F*). The changes of V_J due to the herbicide action occurred again prior to the changes of F_0 and F_V/F_P. An increase of V_J in L1 leaves was observed one day after the application of cyanazine. A maximal increase of V_J (by about 70 %) in the L1 leaves was obtained 12 d after the herbicide application. The real value (median) of this V_J maximum was 0.93. Changes of F_0 and F_V/F_P of the L1 leaves occurred afterwards, they were very expressive (Fig. 3*E,F*) with maximum (F_0) and minimum (F_V/F_P) 5 and 12 d after the application, respectively. The increase of F_0 and decrease of F_V/F_P in these terms of measurement represented more than 100 and 80 % of the control values, respectively. Twenty-one days after the application, the differences in F_0 and F_V/F_P observed between the affected and control plants were reduced which reflected a partial recovery of the plants from the cyanazine action. In contrast to C_{30}, the application of C_{60} caused Chl fluorescence changes also in younger leaves L2 (Fig. 3*D-F*). In these leaves an increase of V_J (by about 40-60 %), a very pronounced increase of F_0 (by about 130 % in maximum), and a decrease of F_V/F_P (by about 20-40 %) were observed in all terms of measurement (*i.e.*, 5, 12, and 21 d after application). The F_0 parameter in the L2 leaves was even higher than that in the L1 leaves (Fig. 3*E*). Also in the L2 leaves a partial recovery of the F_0 and F_V/F_P parameters towards the values of the control plants was recognizable. The fluorescence parameters of the L3 and L4 leaves were not affected by C_{60}.

Table 2. Characteristic extremes (CE) and topographic gradients (TG) of the chlorophyll fluorescence parameters. Determined from the values measured on May 26 (12 d after application of cyanazine; C_{30} and C_{60} = 30 and 60 mg m^{-2}, respectively) and June 4 (21 d after application of cyanazine) 1997 on three leaves (L1-L3) of barley plants, estimated from medians (n = 10).

		12 d after application			21 d after application		
		Control	C_{30}	C_{60}	Control	C_{30}	C_{60}
V_J	CE	0.549	0.541	0.560	0.525	0.525	0.523
	TG	0.054	0.344	0.654	0.106	0.507	0.772
F_0	CE	346	350	374	312	311	340
	TG	0.079	0.803	1.120	0.037	0.432	0.621
F_V/F_P	CE	0.810	0.815	0.800	0.827	0.834	0.825
	TG	0.012	0.206	0.806	0.045	0.073	0.344
F_P	CE	1329	1908	1927	1775	1953	2001
	TG	0.067	0.050	0.689	0.102	0.059	0.286

The characteristic extremes and topographic gradients of the Chl fluorescence parameters determined from the values of three leaves (L1, L2, and L3) 12 and 21 d after the application of cyanazine are shown in Table 2. Based on the introduced interpretation of CE and TG (see Materials and methods), the cyanazine did not affect the youngest plant parts (the L3 leaves—compare with Fig. 3) as indicated by the unchanged CE. However, higher values of the topographic gradient in

comparison with the control plants indicate that an increase of the plant heterogeneity occurred after the application of the herbicide.

The topographic gradient of V_J was higher in the second term (June 4) than in the first one (May 26). On the contrary, TG of the F_0 and F_V/F_P parameters in the plants affected by cyanazine decreased with time reflecting a partial recovery of these parameters in the most affected leaves (see Fig. 3).

Table 3. Dry mass [g] of the above-ground part of plants and spike number per plant determined on July 26, 1997 (growth phase 92 DC - full ripeness). C_{30} and C_{60} = 30 and 60 mg(cyanazine) m^{-2}, respectively. Medians and quartiles (in parentheses) are shown. *,*** significant at $p<0.05$ and $p<0.001$ compared to the control values, respectively.

	Control	C_{30}	C_{60}	*n*
Dry mass of the above-ground part of plant [g]	8.75 (8.73; 8.86)	7.92*** (7.89; 7.97)	7.56*** (7.52; 7.62)	20
Spike number per plant	6.00 (5.50; 6.50)	5.00* (4.00; 6.00)	5.00 (4.00; 6.00)	10

Growth and yield characteristics: The dry mass of the above-ground part of plants and the number of spikes per plant determined in the growth phase of full ripeness were reduced in plants affected by cyanazine (Table 3). The decrease of the dry mass was higher in the case of C_{60}.

The application of C_{30} and C_{60} caused an approximate decrease of 15 % of the total mass of grains in a spike (MG, Fig. 4). However, this decrease was different for various types of stalk. For C_{30}, the decrease of MG of the main stalk was only about 4 %, whereas in the other stalks MG decreased to 82 % of the control value. On the contrary, for C_{60} the determinative decrease of the mass of grains (by about 15 %) occurred in the main stalks (Fig. 4*A*). Further, C_{30} caused a decrease of the number of grains per spike, whereas C_{60} induced a decrease of the caryopsis mass (Fig. 4*B,C*).

Discussion

Dynamic Chl fluorescence response to the herbicide application: The application of *Bladex 50 SC* caused changes of Chl fluorescence (Fig. 3, Table 2) which were connected with the inhibition of electron transport from Q_A^- to Q_B (Brewer *et al.* 1979). Changes of the fluorescence parameters observed within three weeks after the application reflected both the development of the herbicide harmful action and a partial regeneration of the barley plants. We chose the three-weeks period because (*a*) the former experience (Lazár *et al.* 1997, Nauš *et al.* 1997) has shown that cyanazine may have a prolonged effect for many weeks in leaves of cereals, and (*b*) the results observed in the later terms are usually strongly influenced by senescence of the leaves (Matoušková *et al.* 1996).

A similar time course of the Chl fluorescence parameters was found for C_{30} and C_{60}. However, C_{60} induced much more pronounced changes of fluorescence parameters both in the first (L1) and second (L2) leaves with a slower kinetics of regeneration (Fig. 3).

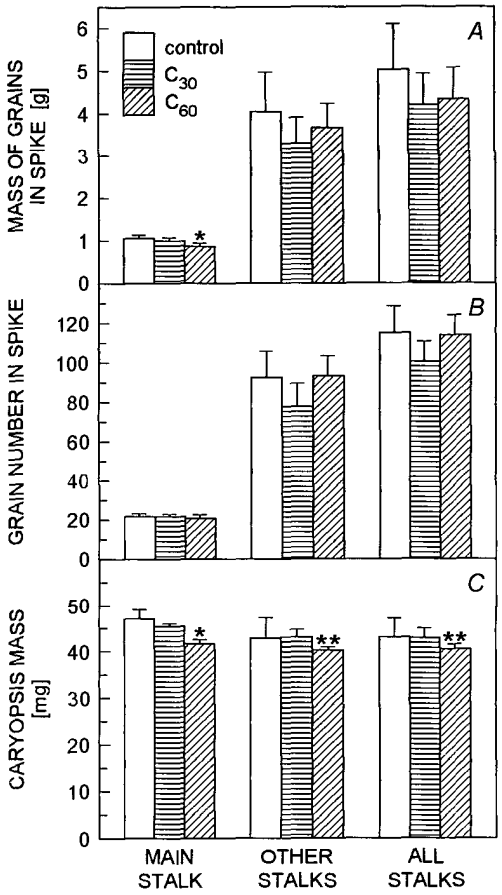

Fig. 4. Effect of cyanazine in doses of 30 and 60 mg m^{-2} (C_{30} and C_{60}) on (*A*) total mass of grains in a spike (MG), (*B*) a grain number in spike (GN), and (*C*) a caryopsis mass (CM). Determined in full ripeness (July 26, 1997). Medians and quartiles are shown, $n = 10$. *,** significant differences at $p<0.05$ and $p<0.01$ compared to the control values, respectively.

Although the reaction of the photosynthetic and cell systems in the leaf to the applied herbicide might be rather complicated, we suggest to distinguish three main phases of the herbicide action characterized by specific changes of the fluorescence parameters:

(*1*) At the early stages of the herbicide penetration (very low herbicide concentration in mesophyll cells) an increase of the J step and hence of the V_J appeared without significant changes in F_0, F_P, and F_V/F_P (a mild effect—see Fig. 2*B*). This situation occurred in the L1 leaves one day after the application of cyanazine (Fig. 3)

or in the L2 leaves 12 and 21 d after the application of C_{30} (Fig. 3*A-C*). This effect has been modelled by an accumulation of the Q_B-nonreducing centres of PS2 (Lazár *et al.* 1997). These centres accept excitation and separate the charges but the electron is transferred only to Q_A (Melis 1991). The accumulation of the Q_B-nonreducing PS2 centres was reported in *Dunaliella salina* in response to sublethal doses of DCMU (Nauš and Melis 1992). Also the Q_B-reducing PS2 centres contribute to the J step (due to a relatively high excitation irradiance), but the effect of increasing amount of the Q_B-nonreducing PS2 centres can be dominant because of the mechanism of PS2-herbicide action.

(*2*) A m e d i u m e f f e c t followed the mild one upon a further penetration of the herbicide molecules into the mesophyll cells. After a distinct V_J increase a strong increase of F_0 (as much as 100 %) followed (Fig. 2*C*). A concomitant slighter increase of F_P appeared (see Table 2, *last row*, CE). As a consequence of this, the value of F_V/F_P decreased by up to 40 %. This was the L1 behaviour after application of C_{30} (Fig. 3*A-C*) and the L2 leaf fluorescence after application of C_{60} (Fig. 3*D-F*). The effect was more profound 5 d after the herbicide application. A hypothesis of an accumulation of highly fluorescing inactive PS2 reaction centres might be one of the explanations of this effect (Lazár *et al.* 1997, Nauš *et al.* 1997, Matoušková *et al.* 1998). We assume that Q_A remained reduced in these centres even after dark-adaptation of leaves, probably due to an inhibition of Q_A^- reoxidation caused by the binding of cyanazine (Velthuys 1981, Vermaas *et al.* 1983).

(*3*) For C_{60} the medium effect in the L1 leaf was replaced by a s t r o n g e f f e c t characterized by a decrease of F_P and F_0. The decline of F_P was stronger than that of F_0 and consequently led to a profound lowering of the F_V/F_P value (see the L1 leaf on the 5th and 12th d after application—Fig. 3*F*). The reason for this decrease is not clear. Both the photoprotective mechanism (xanthophyll cycle) and photoinhibitory PS2 damage can decrease the PS2 Chl fluorescence (Gilmore *et al.* 1996). The xanthophyll cycle mechanism could be excluded, because it requires the formation of a transmembrane difference of pH (Demmig-Adams 1990). At higher herbicide concentrations the formation of ΔpH (Schreiber and Bilger 1987) and de-epoxidation of violaxanthin to zeaxanthin (Barry *et al.* 1990) are inhibited. Thus, the photoinhibitory damage of PS2 as the reason of the observed decrease of F_P seems more probable. The mechanism of photoinhibitory damage may be associated with singlet-oxygen- and oxygen-radical-mediated degradation of pigments and proteins of the PS2 reaction centre (Kirilovsky *et al.* 1994, Andersson and Barber 1996). A pigment breakdown following the PS2 herbicide treatment was reported in intact chloroplasts (Barry *et al.* 1990) and in detached cotyledons (Pallett and Dodge 1980) and was assigned to a photooxidative damage.

The regeneration of the leaf photosynthetic apparatus from the herbicide effect was reflected in a sequence of fluorescence events inverse to that appearing during the development of the herbicide action. The lowering of the F_P decrease during regeneration led to an increase of F_V/F_P (Fig. 3*F*, L1 leaf, 21st d) and the decrease of F_0 led to an increase of F_V/F_P up to the standard value (Fig. 3*B,C*, L1 leaf; Fig. 3*E,F*, L2 leaf).

The processes of regeneration from the herbicide action were complicated by the natural senescence of individual leaves. The natural senescence leads to an increase of V_J followed by an increase of F_0 (see, *e.g.*, Matoušková *et al.* 1996). Many other effects may come into play, *e.g.*, the heterogeneity of the herbicide penetration both on the cell and chloroplast level, induced structural effects (*e.g.*, arrangement of chloroplasts in a cell), *etc.*

The effect of herbicide dose on the crop yield: The fluorescence measurements made on the main stalks of plants have indicated a distinct difference between the changes in thylakoid function caused by C_{30} and C_{60}. Only the L1 leaf, which was fully developed in the time of application, was affected to a medium extent by C_{30} whereas two leaves (L1 and L2) were affected by C_{60} (the L1 leaf was affected strongly). Were the reactions found for the main stalk also reflected in the final crop yield of the barley plants ?

The mass of grains in an individual spike (MG, Fig. 4*A*) is given by a product of grain number (GN, Fig. 4*B*) and caryopsis mass (CM, Fig. 3*C*). The application of the herbicide caused a decrease of MG by about 14 % (although this decrease was not statistically significant at $p>0.05$). Surprisingly, this effect was similar for C_{30} and C_{60} if all productive stalks were considered. However, inspecting Table 3 and Fig. 4, two different reactions of the whole plant can be inferred. The barley plant is composed of the main stalk, and other younger stalks developed within the same plant in the course of tillering. Several stalks within the plant reach the full development and provide the spike, other stalks are not productive.

At C_{30}, when the changes of Chl fluorescence signalled a slighter damage of photosynthetic apparatus, the plants tended to support the older parts of the plant (the main stalk) by repair processes at the expense of the younger stalks. The quantities MG, GN, and CM were rather conserved in the main stalk whereas MG and GN decreased in other stalks. A retardation in ontogeny of these stalks lowered mostly the grain number in a spike (Fig. 4*B*).

The effect of C_{60} was different: the Chl fluorescence parameters reflected a more pronounced and longer herbicide action. The senescence of the older part of plant was accelerated and the assimilates were preferentially allocated into the younger stalks. The GN (Fig. 4*B*) was the same for this variant and for the control plants. This effect is usually characterized as a rejuvenation and it is initiated by other stronger stresses. We suppose that this effect was also induced by C_{60}.

Conclusion: The present study indicates that the simple, rapid, and non-destructive method of Chl fluorescence topography can be successfully used for monitoring of the PS2 herbicide effect on the field-grown barley plants. The analysis of changes of Chl fluorescence parameters informs on the level of damage to the photosynthetic apparatus and the ability of the plants to cope with the herbicide effect. We suggest that the specific changes of the individual parameters of the O-J-I-P transient can predict the further development of different plant parts.

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