

TEMPERATURE AFFECTS HERBICIDE-SENSITIVITY OF PEA PLANTS*

Petar Lambrev, Vassilij Goltsev^{1**}

Department of Biophysics and Radiobiology, Biological Faculty, St. Kliment Ohridski University of Sofia, 8 Dragan Tsankov Blvd., 1421 Sofia, Bulgaria

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Summary. The effect of temperatures in the range of 5 to 45°C on the kinetics of prompt and millisecond delayed fluorescence of herbicide-treated pea leaves was studied. The leaves were treated for 2 h at 22°C with herbicide solutions of diuron, atrazine and dinoseb, belonging to two super-families – urea/triazines and phenolic herbicides. Luminescence characteristics were registered at different temperatures after 3 min preincubation of the leaves in a dark measuring chamber. Temperatures of about 30–35°C were found to be optimal for photosynthetic electron transport giving highest levels of delayed fluorescence emission and variable fluorescence. The effect of diuron and atrazine was found to be negligible at low temperatures (5–10°C) and expressed in greater extent at elevated temperatures (up to 45°C). Dinoseb induced strong inhibiting effect at all temperatures. A conformational change in the D1 protein was suggested regarding the affinity of the herbicide molecules towards the Q_B-binding site, in a way that the herbicide can not compete with the plastoquinone at low temperatures and is readily displaced.

Key words: delayed fluorescence, electron transport, herbicides, high and low temperature, *Pisum sativum* L., variable chlorophyll fluorescence

Abbreviations: DF – delayed fluorescence; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_o – constant (ground) fluorescence; F_v – variable fluorescence; F_m – maximal fluorescence; F_s – quasi stationary fluorescence level; I₁₋₄ and D_{2,5} – parameters of the delayed fluorescence induction kinetics; PF – prompt fluorescence; PQ – plastoquinone; PSI – Photosystem 1, PSII – Photosystem 2; PSA – photosynthetic apparatus

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** Corresponding author. E-mail: goltsev@biofac.uni-sofia.bg

Introduction

Herbicides are a backbone component of modern agriculture in developed countries. Their use has resulted in dramatic increases of crop productivity over the last four decades. Herbicides modify the cell cycle, plastid metabolism, or they act as endogenous growth regulators, but for the most part they affect the photosynthetic reactions – dark reactions or photo-induced electron transport (electron transport inhibitors, artificial acceptors and inhibitors of ATP synthesis) (Percival and Baker, 1991). A cardinal site of inhibition of the photosynthetic electron transport is the PSII donor side. The mechanism of inhibition stands on a competitive binding of the herbicide to the Q_B -site on D1-protein in the reaction centre of PSII (Velthuys, 1981; Lavergne, 1982; Vermaas et al., 1984; Trebst, 1987) thus preventing Q_A^- to reduce Q_B . Two amino acid residues binding with the quinone molecule in the region of D1 exist – Ser-264 and His-215. Herbicides are divided into two types in respect to their corresponding binding amino acid as well as to their structure – (these are) the urea/triazine type and the phenol type respectively (Trebst, 1987; Oettmeier, 1992). The former includes the herbicides diuron and atrazine which bind to Ser-264. Dinoseb belongs to the latter, phenol type. In contrast to the electrostatic interactions between plastoquinone and D1, the herbicide binding mechanism involves forming of hydrogen bonds due to sterical correspondence between herbicide molecules and the Q_B -site (Mets and Thiel, 1989; Fuerst and Norman, 1991).

The prompt and delayed chlorophyll fluorescence kinetic measurements are non-invasive and highly informative methods for monitoring the state of PSII and the photo-induced electron transport (Duysens and Sweers, 1963; Havaux and Lannoye, 1983). From the induction kinetic curves of prompt fluorescence one can estimate the photosynthesis quantum yield, fluorescence quenching, size and redox state of the PQ pool (Lavergne, 1974; Buttler, 1977; Krause and Weis, 1984). DF allows assessing the influence of environmental stress on the overall process of photosynthesis (Havaux and Lannoye, 1983; Yordanov et al., 1987; Bilger and Schreiber, 1990). Induction curves of DF are assumed to be good indicators for the electron transport capacity (Schreiber and Schliwa, 1987; Bilger and Schreiber, 1990) and the proton gradient across the thylakoid membranes (Havaux and Lannoye, 1983; Fork et al., 1985; Bilger and Schreiber, 1990; Härtel et al., 1993). Thus, they provide information about energization and the integrity of thylakoid membranes (Havaux and Lannoye, 1983; Fork et al., 1985; Bilger and Schreiber, 1990).

The photosynthetic apparatus is the most heat-sensitive component of plants. Temperature modifies the physicochemical properties of the thylakoid membrane – permeability, fluidity, lipid and protein complex mobility (Schreiber and Berry, 1977), as well as the electron transport rate. It is established that within the electron transport chain PSII is most vulnerable to temperature, on its donor side, as well as on its accep-

tor side, that indicated by its induction kinetics (Krause and Santarius, 1975; Havaux et al., 1991). It was suggested that 2 of the 4 Mn atoms in the oxygen-evolving complex could be split off. High temperatures lead to dissociation of the light-harvesting complex of PSII (Armond and Schreiber, 1978; Gounaris et al., 1984).

Temperature-induced changes in PSII influence the induction kinetics of PF and DF (Krause and Weis, 1984; Schreiber et al., 1988; Larcher et al., 1990).

Since beyond-optimal temperatures alter membrane protein conformation, along with the PSII reaction centre proteins, it could be assumed that such temperatures would change the ability of D1 to bind herbicides (Vasil'ev and Venediktov, 1993).

In the present work the effect of temperatures in the range of 5–45°C on the kinetic characteristics of prompt and delayed fluorescence of pea leaves treated with herbicides was investigated, in order to clarify how temperature determines the sensitivity of PSII to herbicides.

Materials and Methods

Objects of experiments were leaves from 14-days pea plants (*Pisum sativum* L.) grown hydroponically in Knopp solution at temperatures of 23–25°C and illumination of 45 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

The following solutions were used for object treatment:

- 1) Control – distilled water;
- 2) DCMU – 10^{-5} M in distilled water, made out of stock substance 3-(3,4-dichlorophenyl)-1,1-dimethylurea, SIGMA, min. 98%;
- 3) Atrazine – 10^{-5} M in dist. water, made out of 2-ethylamino-4-chloro-6-isopropylamino-1,3,5-triazine, SERVA, p.a.;
- 4) Dinoseb – 10^{-4} M in dist. water, made out of 6-(sec-butyl)-2,4-dinitrophenol, SERVA, p.a.

Groups of about 20 leaves were arranged in Petry dishes over 10 ml treatment solution and incubated at 22°C for 2 hours (the 2nd hour in dark for PSA adaptation).

Prompt and delayed fluorescence induction kinetics measurements were conducted using a fluorometer FL2006 (Test, Russia) as in Goltsev and Yordanov (1997).

The whole experiment took place in a dark room. Before measuring the leaves were tempered in the measuring chamber of the phosphoroscope at the desired temperature for 2 min. Measurements were conducted at temperatures in the range 5–45°C at intervals of 5°C. Induction kinetics of prompt and delayed fluorescence were registered simultaneously for 3 min.

At least 3 repetitions were accomplished for each experiment. Dispersion analysis was applied to the data obtained.

Results

The induction kinetics of PF and DF, recorded at temperatures in the range of 5–45°C are represented on Fig. 1. The curve's shape as well as the overall intensity change with temperature.

With the temperature increase, the overall intensity of DF monotonously increased to maximal values at 30°C. The increase was more obvious during the slow phase, representing the generation of membrane electrochemical potential. At the same time, considerable changes in the fast phase were observed – at low temperatures I_1 predominates over I_2 , the opposite is valid over 15°C. The time, required for reaching the maximal values in the DF slow phase, monotonously decreased with temperature. This may be due to the acceleration of the photosynthetic processes.

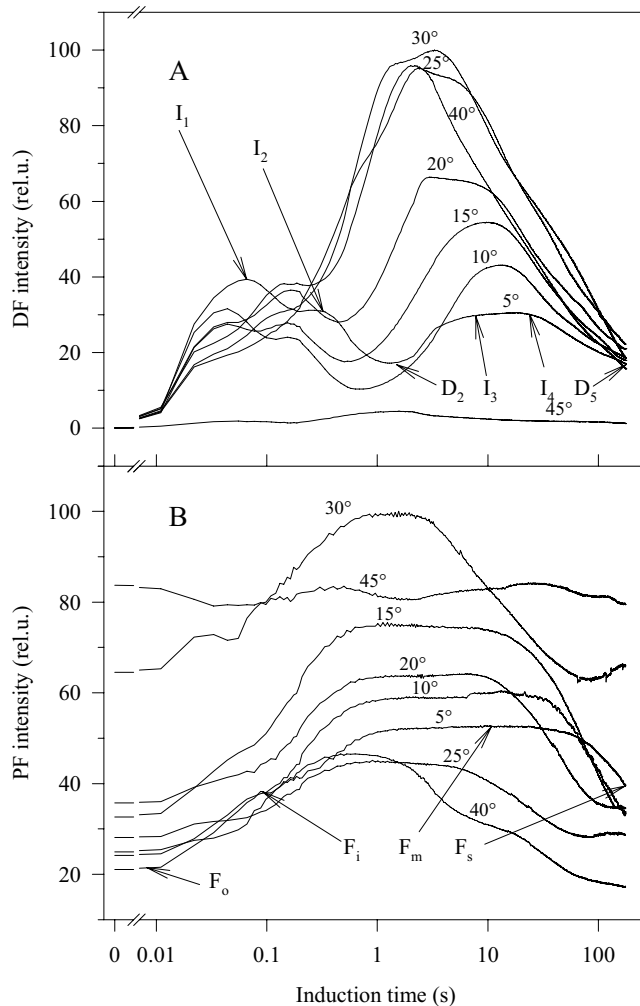


Fig. 1. Induction kinetics of prompt and delayed fluorescence of control pea leaves, measured at different temperatures in the range of 5 to 45°C.

A – Delayed fluorescence;
B – Prompt fluorescence.

Prior to registration the leaves were kept in distilled water for 2 h (1 h in light followed by 1 h in dark) and 3 min in the measuring chamber at the corresponding temperature. The actinic light intensity at the sample level was $1.2 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. I_1 , I_2 – first and second maximums of DF; D_2 – first minimum after I_2 ; I_3 , I_4 – slow maximums of DF; D_5 – DF level 180 s after illumination (steady state). F_0 – ground fluorescence (extrapolated); F_i – first maximum of PF; F_m – maximal fluorescence; F_s – PF level 180 s after illumination.

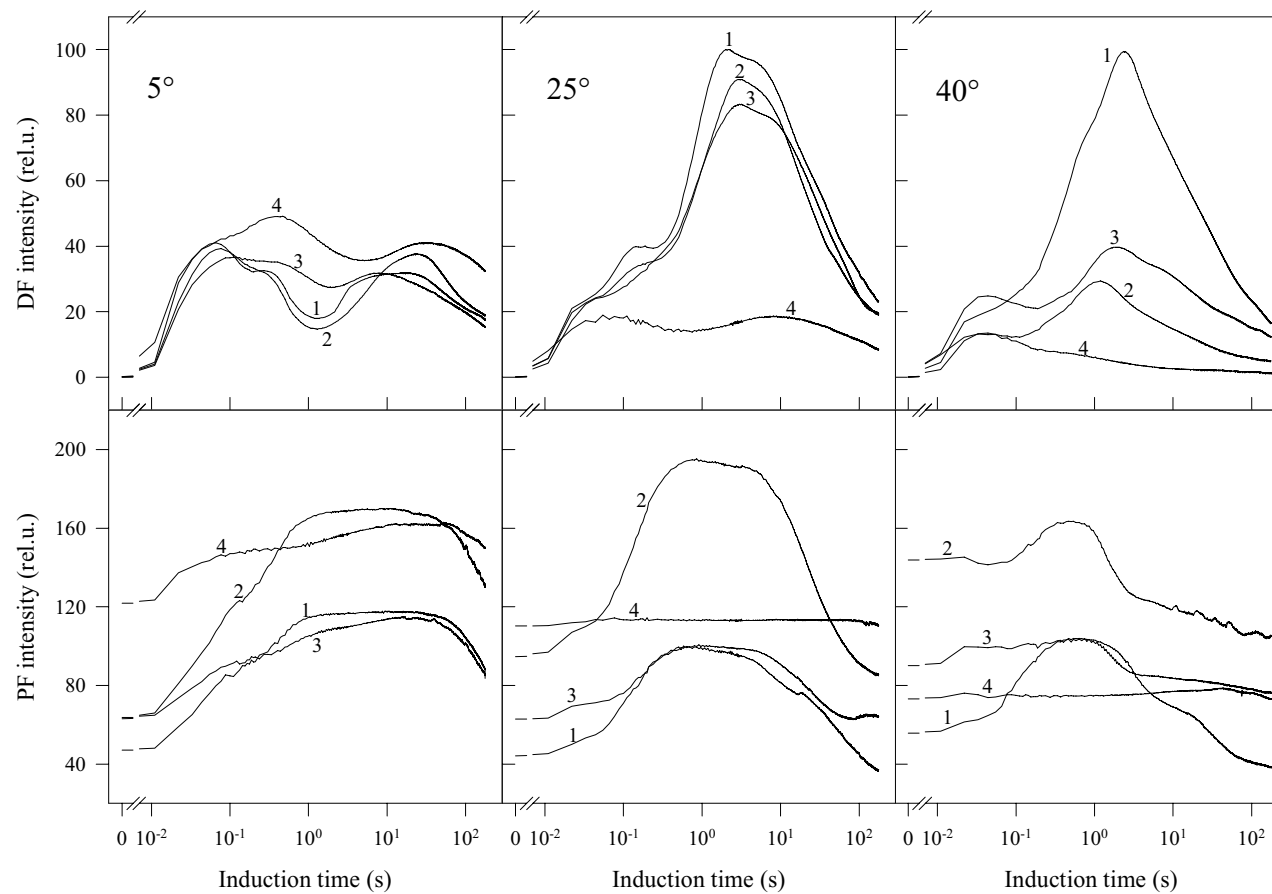


Fig. 2. Induction kinetics of prompt (lower plots) and delayed (upper plots) fluorescence of pea leaves treated for 2 h (1 h in light followed by 1 h in dark) with 10^{-5} M DCMU, 10^{-5} M atrazine or 10^{-4} M dinoseb respectively, measured at 3 different temperatures. 1 – control, 2 – DCMU, 3 – atrazine, 4 – dinoseb. The experimental procedure was as in Fig. 1.

Temperature treatment affected the characteristics of PF (Schreiber and Bilger, 1987; Larcher et al., 1990). Elevated temperatures diminish the variable fluorescence. Temperature of 45°C declines any variable fluorescence and fluorescence quenching, while the level of dead fluorescence F_0 is considerably raised (Bilger et al., 1987; Goltsev et al., 1994).

The two-hours incubation of leaves in herbicide solution modified the induction kinetics in shape and scale, depending on temperature and herbicide applied (Fig. 2). DCMU effect appears at temperatures of 35–40°C, atrazine – $T \geq 25^\circ\text{C}$, while dinoseb acts practically throughout the entire temperature range.

The DF induction kinetics of DCMU treated leaves followed the control kinetics. Apparent differences are noted during the slow induction phase. At low temperatures, the inhibitory effect is insignificant, but increases with the further rise in the temperature and is highest at 40°C.

Atrazine's inhibitory effect was more pronounced than that of DCMU. The major differences with the control are the lower level of I_1 and the considerably higher D_2 . Dinoseb treatment strongly reduces the overall intensity of DF at all temperatures and the curve eventually becomes monophasic at 40°C with a single peak – I_1 .

At low temperatures the shape of the PF induction curves of atrazine and DCMU treated probes was also alike to the control. The level of constant fluorescence F_0 remains unchanged, as opposed to dinoseb, which shows a noticeably higher F_0 . At elevated temperatures F_0 increases and the variable fluorescence tends to decrease. The slow phases of the induction curves, reflecting the photoinduced fluorescence quenching, are not affected, which leads to the assumption that the dark phase of the photosynthesis remains unmodified.

The temperature dependence of the sensitivity to herbicide treatment was confirmed by the analysis of some given parameters, estimated from the induction kinetics of PF and DF (Fig. 3–6).

The maximal DF intensity I_4 smoothly increased in the range of 5–30°C and was 3 fold higher at 30°C than at 5°C (Fig. 3). A steep decline in the intensity is observed over 30°C. DCMU and atrazine do not modify this parameter at low temperatures, DCMU exhibits a considerable inhibitory effect at 40°C, atrazine – in the range of 25–40°C. The maximal DF intensity of the dinoseb-treated leaves is insignificant as compared to all remaining probes in the temperature range.

The ratio of the maximums in the fast phase of DF induction – I_1/I_2 – decreased with temperature and reached its minimal values at 35°C (Fig. 4). Further increase of temperature gives rise to I_1/I_2 , conforming to the maximal inhibition of the electron transfer. The effect is observed with control as with herbicide treated leaves.

The relative variable fluorescence – F_v/F_0 gradually decreased with temperature to a total decline at 45°C (Fig. 5), as shown by the results of Georgieva and Yordanov (1993). Herbicides decrease the value of F_v/F_0 , dinoseb being most active, followed by atrazine. The action of atrazine and dinoseb is pronounced in the entire tempera-

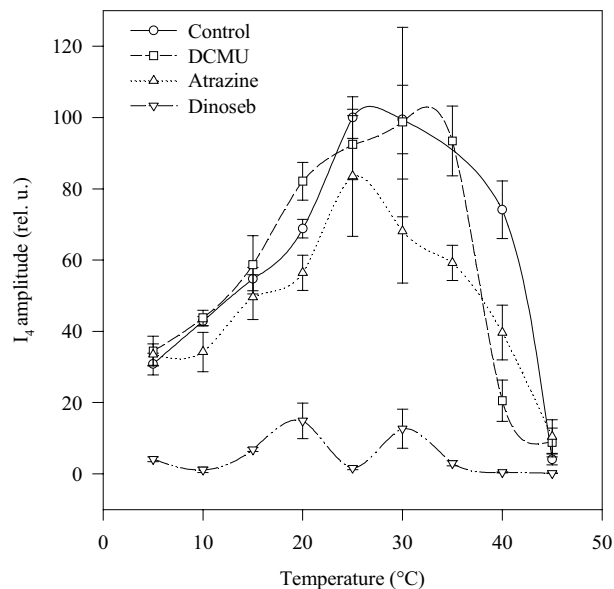


Fig. 3. Effect of measuring temperature on the amplitude of the delayed fluorescence induction maximum I_4 in pea leaves treated by herbicides. The experimental conditions as in Fig. 2.

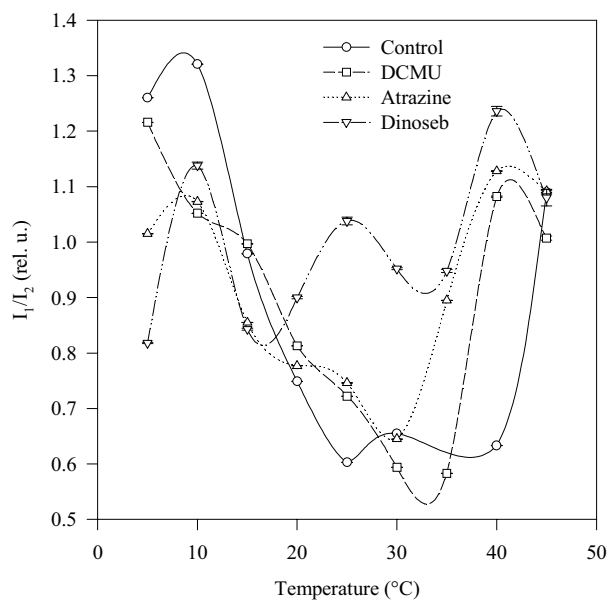


Fig. 4. Effect of measuring temperature on the ratio of the delayed fluorescence induction maximums I_1/I_2 in pea leaves treated by herbicides. The experimental conditions as in Fig. 2.

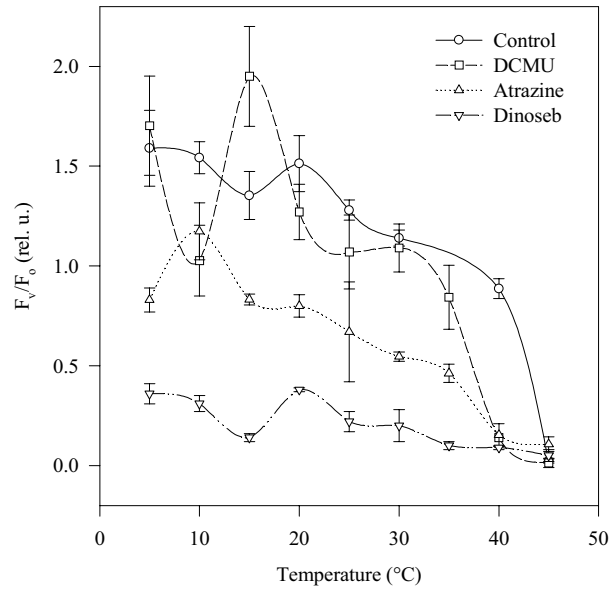


Fig. 5. Effect of measuring temperature on the prompt fluorescence relative parameter F_v/F_o in pea leaves treated by herbicides. The experimental conditions as in Fig. 2.

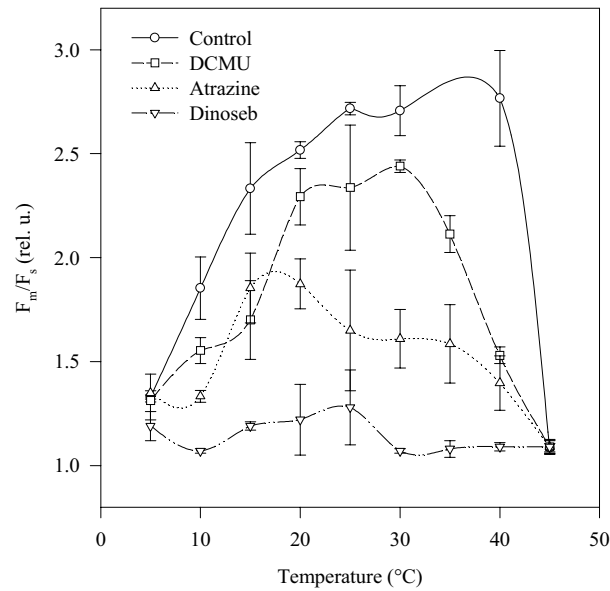


Fig. 6. Effect of measuring temperature on the prompt fluorescence relative parameter F_m/F_s in pea leaves treated by herbicides. The experimental conditions as in Fig. 2.

ture range, but is emphasized at higher temperatures. DCMU provokes no significant differences with the control below 35°C, its effect appears at 40°C.

The temperature dependence of the F_m/F_s ration showed a maximum at around 35°C (Fig. 6). The maximal value for DCMU is at 30°C, for atrazine and dinoseb – between 15 and 20°C. The herbicide effect is clear in the range of 10–40°C.

Discussion

Temperature, as an environmental factor with a broad range of activity, simultaneously modifies the rates of virtually all photosynthetic reactions. Measuring the luminescent characteristics of plants one can carry out a qualitative assessment of the temperature effect over different stages in the process of light energy transformation.

The effect of temperatures between 5 and 45°C is diverse. The photosynthetic activity of the pea plants shows a general increase with increasing temperatures up to an optimal temperature of about 35°C (Ludlow and Wilson, 1971; Larcher et al., 1990) (Fig. 1). At temperatures around 40–45°C the normal functioning of the PSA is greatly impaired (Bilger et al., 1987; Bukhov et al., 1987; Larcher et al., 1990).

In the range of 5 to 35°C, the following changes in the PSA are observed by means of luminescent characteristics' measurement:

a) Acceleration of the electron transport between the $Q_A Q_B$ complex and the PQ pool, which is confirmed by the rise in the level of I_2 at expense of I_1 (Fig. 1 and 4) (Goltsev and Yordanov, 1997);

b) Acceleration of the intersystem electron transfer and a relatively low redox state of PQ pool. This explains the rise of DF intensity at the D_2 minimum (Fig. 1), the increased thylakoid membrane energization, and the slow rise of the DF induction kinetic to I_4 (Fig. 3);

c) Intersystem electron transfer is disturbed at unfavorable temperatures, which is evident from the DF maximums ratio, I_1/I_2 (Fig. 4). It may be a result of D1 protein conformational changes having an effect on its interactions with the PQ molecule. Probably, at temperatures between 20 and 35°C the binding constant is optimal for electron transfer through the Q_B site.

d) Temperature induces general acceleration of the photosynthetic processes resulting in reaching earlier DF maximums (Fig. 1A) and earlier engagement of the PF quenching (Fig. 1B). Similar temperature effect was observed with barley leaves (Zaharieva et al., 1999).

e) Faster activation of PSI and Calvin cycle reactions – apparent from the depth of the I_4 – D_5 drop of DF and the quenching of PF (F_m – F_s) (Fig. 1).

At temperatures of 40–45°C the electron transport is inhibited, which leads to lowered levels of DF (Bukhov et al., 1987) and variable fluorescence (Larcher et al., 1990). The raised constant fluorescence level F_0 suggests a dissociation of the light-

scattering complex of PSII (Armond and Schreiber, 1978) or an inhibition of the electron transport in the acceptor complex of PSII (Bukhov et al., 1993). According to Laasch (1987), Santarius and Weis (1988) the reduced variable fluorescence is due to a limitation of electron donation to PSII.

The chosen herbicide concentrations and treating method indicate that dinoseb is the most efficient among the three herbicides (dinoseb's concentration surpassed the others by one order). DCMU and atrazine act in a lesser degree, the effect being greatly dependent on the momentary object temperature. DF inhibition efficiency of the applied herbicides is maximal for DCMU and minimal for dinoseb, in the case of isolated chloroplasts (Doltchinkova et al., 1997). Native objects differ from thylakoid membranes showing less sensitivity to DCMU and atrazine and higher sensitivity to dinoseb (Laasch, 1987).

Herbicide effect was less pronounced at low temperatures as estimated by variable (Fig. 2 and 3) as well as delayed fluorescence (Fig. 2 and 5). The reduced I_2-D_2 drop is an evidence of the lowered ability of the reaction centres to transfer electrons to PQ. The remaining noninhibited by herbicide reaction centres are oxidized by the same PQ pool and are able to maintain lower redox level. Herbicide action increases with temperature and is observed in greater extent in the range of 35–44°C.

Within the temperature range optimal for photosynthesis (20–35°C) the herbicides provoke decrease in the electron flow as well as in the transmembrane gradient (suppressing I_4 , I_5). In presence of DCMU the photoinduced fluorescence quenching does not decrease considerably until 30°C. Atrazine suppresses this property over 20°C, and dinoseb – at all temperatures. May be the dark phase of photosynthesis remains unaltered in treated objects, but the share of energetic quenching, depending of intersystem transfer rate, decreases.

Electron transport is severely suppressed at 40°C, which shows that DCMU and atrazine inhibit very effectively at these temperatures.

We suppose that the change in the herbicide sensitivity of PSII to DCMU and atrazine is related to temperature-induced conformational transitions in the D1 protein of the reaction centre, altering the affinity of the Q_B pocket to the corresponding herbicide molecule (Vasil'ev and Venediktov, 1993). At elevated temperatures the conformation of the herbicide-binding site in the reaction centre of PSII is optimal for providing interactions with the herbicide molecule. At low temperatures (5–15°C) the conformation of D1 determines low affinity for both the PQ and the herbicide, hence they are not competitive to PQ at their low concentrations.

The absence of a temperature effect on dinoseb efficiency is probably related to interactions of dinoseb with His-215, located within the hydrophobic part of the thylakoid membrane, in contrast of Ser-264, that forms H-bonds with diuron and atrazine molecules in hydrophilic part (zone) of the thylakoid membrane (Trebst, 1987; Draber et al., 1995).

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