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Comparative thermoluminescence study of triazine-resistant and -susceptible biotypes of *Erigeron canadensis* L.

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By means of thermoluminescence measurements, the midpoint oxidation-reduction potentials of the primary (Q_A) and secondary (Q_B) quinone electron acceptors of Photosystem II, as well as the equilibrium constant of the equilibrium $Q_A^- \cdot Q_B \overset{K}{\rightleftharpoons} Q_A \cdot Q_B^-$, were compared in chloroplasts of triazine-susceptible and triazine-resistant biotypes of *Erigeron canadensis* L. It was observed that at pH 7.5 in DCMU-treated chloroplasts the main thermoluminescence band attributed to radiative charge recombination of the $S_2Q_A^-$ redox state appeared at about the same temperature (approx. 10°C) in both biotypes, indicating that the triazine resistance is not accompanied by an alteration in the midpoint potentials of the S_2/S_3 and Q_A/Q_A^- redox couples. On the other hand, the peak position of the thermoluminescence band associated with the $S_2Q_B^-$ state was shifted from 32 to 15°C in the glow curve of untreated resistant chloroplasts as compared to the sensitive ones, suggesting a decrease in the midpoint potential of the Q_B/Q_B^- redox couple in resistant chloroplasts. Computer-assisted analysis of the thermoluminescence measurements showed that the redox distance between Q_A and Q_B was 71 mV in sensitive chloroplasts, and about 32 mV in resistant ones. This corresponded approximately to a change in the value of the equilibrium constant between $Q_A^- \cdot Q_B$ and $Q_A \cdot Q_B^-$ from 16 to 3.5 in sensitive and resistant thylakoids, respectively. The semiquinone equilibrium between Q_A and Q_B was found to be greatly dependent upon pH. Decrease of the pH from 7.5 to 6.0 resulted in a 2.8-fold increase in the value of the equilibrium constant, from 16 to 44, in sensitive chloroplasts. The shift in the peak position of the 32°C thermoluminescence band (and consequently in the redox potential of the Q_B/Q_B^- couple) was also observed in three other triazine-susceptible/resistant biotype pairs. However, the extent of the shift varied with the plant species investigated. From the results of thermoluminescence measurements it is concluded, in agreement with the hypothesis of other authors, that the lowering of the redox potential of the Q_B/Q_B^- couple compared to that of the Q_A/Q_A^- couple is probably a trait characteristic of the triazine-resistant biotypes.

Introduction

In recent years, biotypes of several weed species resistant to *s*-triazine herbicides have been reported (for review, see Ref. 1). The resistance to

Abbreviations: Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; PS, Photosystem; Q_A, primary quinone electron acceptor of Photosystem II; Q_B, secondary quinone electron acceptor; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

inhibition of electron transport by triazine herbicides is due to an alteration in the herbicide/quinone binding environment, which also binds the secondary quinone acceptor, Q_B. The membrane alteration responsible for the decreased herbicide affinity at the triazine-binding site is accompanied by a change in the functioning of electron carriers on the reducing side of PS II. Bowes et al. [2] demonstrated that electron transfer from the primary quinone to the secondary quinone acceptor was at least 10-times slower in chloroplasts from triazine-resistant plants. Fluorescence induction transients observed upon illumination of dark-adapted chloroplasts indicated that at the onset of illumination a higher initial concentration of reduced primary quinone, Q_A^-, accumulated in chloroplasts of resistant weed biotypes compared to susceptible weed biotypes [3–5]. The damping of the oscillation in O_2 flash yield, which was observed to be much higher in resistant thylakoids than in susceptible ones, also suggested an increased equilibrium concentration of Q_A^- in the resistant thylakoids [6–8]. In triazine-resistant *Amaranthus hybridus* 25% of the PS-II reaction centers were found to be in a photochemically inactive state [9]. It has been proposed that this quantum inefficiency can be accounted for by a modest decrease in the equilibrium constant between the quinone acceptors in the resistant biotype. Vermaas and Arntzen [4] concluded that the increased equilibrium concentration of reduced Q_A in resistant chloroplasts as compared to susceptible ones could be explained both by a decrease in the affinity of plastoquinone for the quinone binding site and by a decrease in the midpoint redox potential of the Q_B/Q_B^- couple.

It has been demonstrated that the redox states of the primary and secondary quinone acceptors of PS II can be investigated by thermoluminescence measurements [10,11]. In the glow curve of chloroplasts excited at $-70°C$ the main thermoluminescence band appearing at about $+30°C$ can be attributed to charge recombination of the $S_2Q_B^-$ state (B band) [10,11]. In the presence of PS-II inhibitors electron transfer between Q_A and Q_B is blocked and the thermoluminescence band peaking at about $+10°C$ is generated by charge recombination of the $S_2Q_A^-$ state (designated either Q [11] or D band [12]). Since any change in the redox state of the Q_A and Q_B acceptors is reflected in the positions of the Q and B bands, thermoluminescence seems to be a suitable method for studying possible modifications in the redox states of Q_A and Q_B which are conferred by herbicide resistance.

In this paper we report on a comparative thermoluminescence investigation of triazine-resistant and susceptible biotypes of *Erigeron canadensis*. The observations clearly demonstrate that the midpoint oxidation-reduction potential of the secondary quinone electron acceptor is lowered in triazine-resistant as compared to triazine-susceptible chloroplasts. The extent of the shift in the midpoint potential of the Q_B/Q_B^- redox couple is estimated from mathematical analysis of the thermoluminescence data. Furthermore, the equilibrium constant is determined for the reaction $Q_A^-Q_B \overset{K}{\rightleftharpoons} Q_AQ_B^-$ in both biotypes.

Materials and Methods

Plant material and isolation of chloroplasts

Seeds of triazine-resistant and triazine-susceptible *Erigeron canadensis* L., *Amaranthus retroflexus* L. and *Solanum nigrum* L. were kind gifts from Dr. P. Solymosi (Research Institute for Plant Protection, Budapest, Hungary), Dr. G. Vytautas (Research Institute for Heavy Chemical Industries, Veszprém, Hungary) and Dr. J. Gasquez (I.N.R.A., Laboratoire de Malherbologie, B.P. 1540, 21034 Dijon Cedex, France), respectively. The TBR2 mutant was selected for terbutryn resistance by A. Cséplö and P. Medgyesy (Institute of Plant Physiology, Biological Research Center, Szeged, Hungary) from a protoplast culture of *Nicotiana plumbaginifolia*.

Chloroplasts were isolated from 2–3-months old, greenhouse-grown seedlings, according to the method described previously [13,14]. For the measurements of fluorescence induction and thermoluminescence, chloroplasts were suspended in a medium containing 0.4 M sorbitol/10 mM NaCl/1 mM $MnCl_2$/5 mM $MgCl_2$/2 mM EDTA/50 mM Hepes at pH 7.5.

Measurements of photosynthetic oxygen evolution

The rate of photosynthetic oxygen evolution

was measured at saturating light intensities using a Clark-type electrode in a temperature-controlled cell at 25°C. The assay medium containing 0.1 M sorbitol/10 mM K_2HPO_4/20 mM NaCl/4 mM $MgCl_2$/2 mM EDTA/50 mM Hepes (pH 7.5)/2 mM ferricyanide and chloroplasts containing 50 μg Chl in a final volume of 3.0 ml [15].

Measurement of fluorescence induction

For the measurement of fluorescence induction transients, a dark-adapted chloroplast suspension containing 10 μg Chl/ml was illuminated by a 450 W xenon lamp through a Corning CS 4-96 glass filter on the opening of a shutter (Uniblitz Model 26L2A0X5; Vincent Associates). The intensity of the homogeneous excitation beam was 10 $W \cdot m^{-2}$. Fluorescence light emitted by the sample was detected by an EMI 9558B photomultiplier placed perpendicular to the excitation beam. The scattered actinic light was filtered out with a Corning 2-64 filter placed in front of the photomultiplier. The fluorescence transients were recorded by a multichannel analyser (ICA 70 KFKI) or by a storage oscilloscope (Philips PM3310).

Measurement of thermoluminescence

Thermoluminescence was measured as described in Ref. 11. 0.4 ml aliquots of dark-adapted samples containing 50 μg Chl/ml were excited by white light (10 $W \cdot m^{-2}$) for 30 s at −70°C. After excitation of the samples, thermoluminescence was measured at a heating rate of 20°C/min. Inhibitors were added at room temperature and the samples were incubated in the dark for 5 min before thermoluminescence measurements. In order to prevent the distortion of the glow curves by the solid-liquid phase transition of water the samples contained either 30 or 60% of glycerol [16].

Mathematical analysis of thermoluminescence bands

A least-squares curve-fitting program was used to fit a single Randall-Wilkins band to the main thermoluminescence band of the glow curves of untreated and DCMU-treated chloroplasts, and the free energy of activation was calculated as previously described [16]. In the knowledge of the activation free energies, the equilibrium constants were calculated as in Refs. 11 and 17. DeVault et al. [17] recently demonstrated that the distribution of electrons over two or more electron carriers (e.g., equilibrium $Q_A^- Q_B \overset{K}{\rightleftharpoons} Q_A Q_B^-$) affects the apparent activation parameters (activation energy and activation entropy) calculated from photosynthetic glow curves. In order to determine the error introduced by using the unmodified Randall-Wilkins theory in the analysis of thermoluminescence, model calculations were carried out. A series of glow curves were simulated according to the modified Randall-Wilkins theory [17], considering various equilibrium constants between Q_A and Q_B. The calculated glow curves were fitted by the unmodified Randall-Wilkins equation applied in our curve analysis [16]. The free energies of activation and the equilibrium constants obtained with the curve resolution technique were compared with the input data. It was found that curve fitting with a Randall-Wilkins equation resulted in a slightly higher free energy of activation for a thermoluminescence band and a slightly higher equilibrium constant between Q_A and Q_B than the prefixed values used in the curve-simulation procedure. This alteration from the prefixed values increased with decreasing equilibrium constant be-

TABLE I

THERMOLUMINESCENCE CHARACTERISTICS AND THE EQUILIBRIUM CONSTANT BETWEEN Q_A AND Q_B IN CHLOROPLASTS OF *E. CANADENSIS* BIOTYPES

Characteristics of the thermoluminescence bands, shown in the upper part of Fig. 2, were obtained by a least-squares fitting of the glow curves [16]. The equilibrium constants were calculated as in Ref. 17. T_m, temperature at the maximum of a thermoluminescence band; ΔF, free energy of activation; K, equilibrium constant between the primary (Q_A) and secondary (Q_B) quinone electron acceptors.

Designation of bands	T_m (°C)	ΔF (25°C) (eV)	K
Susceptible chloroplasts (pH 7.5)			
B (no addition)	32	0.850	16
Q (+DCMU)	9	0.779	
Resistant chloroplasts (pH 7.5)			
B (no addition)	15	0.809	3.5
Q (+DCMU)	7	0.777	

tween Q_A and Q_B. For susceptible chloroplasts ($K = 16$) the alteration was completely negligible and in the case of resistant chloroplasts ($K = 3.5$) it was about 30%. Therefore, in the case of sensitive chloroplasts the free energies of activation and the equilibrium constants (Table I) obtained by application of the Randall-Wilkins equation in the analysis of our thermoluminescence measurements can be considered good approximations to the true values. However, for resistant chloroplast the calculated value of the equilibrium constant is higher than expected [5,9] and is probably only a rough approximation of the actual value.

Results and Discussion

To characterize the degree of atrazine resistance in the *E. canadensis* biotype, inhibition of the Hill reaction from H_2O to ferricyanide was mesured in the presence of increasing concentrations of atrazine, and the I_{50} values (concentration of herbicide giving 50% inhibition of electron transport) were determined for both susceptible and resistant chloroplasts. The I_{50} values were 160 nM and 120 μM for atrazine in susceptible and resistant biotype chloroplasts, respectively. The resistance ratio (I_{50} value of resistant/I_{50} value of susceptible) was about 750, which is comparable with the resistance ratio (1000) found for *A. retroflexus* L. [3].

It has been observed that chloroplasts isolated from triazine-resistant and susceptible plants display greatly different fluorescence induction transients [18,19]. The intermediate level of the fast-rising transient portion of the fluorescence was higher for resistant chloroplasts than for sensitive ones. This observation can be explained by assuming that in dark-adapted triazine-resistant thylakoids the initial concentration of Q_A^- is higher upon illumination, due to the lowered midpoint redox potential of the Q_B/Q_B^- couple [4,5]. In agreement with the results obtained for other triazine-susceptible/resistant biotype pairs, resistant *E. canadensis* chloroplasts exhibited a higher intermediate (F_i) level of fluorescence than the susceptible chloroplasts (Fig. 1). The 'original' level of fluorescence observed immediately after the onset of illumination (F_0) and the maximum level of fluorescence (F_m) were nearly equivalent in both induction curves. Consequently, the quickly rising fast

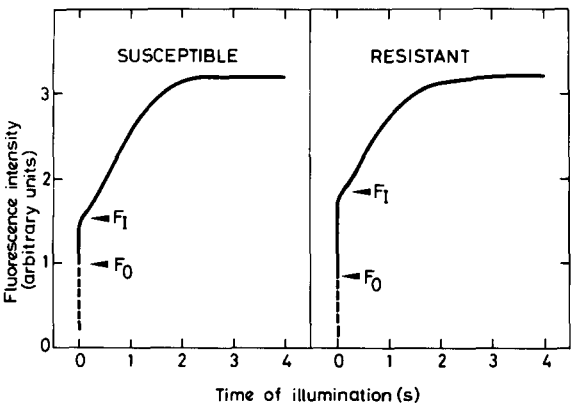

Fig. 1. Chlorophyll fluorescence transient changes observed upon illumination of dark-adapted chloroplasts isolated from triazine-susceptible and resistant biotypes of *E. canadensis* L. F_0 levels of fluorescence were determined within 5 ms after full shutter opening. The F_1 level of fluorescence in untreated samples was obtained from the fluorescence intensity after 50 ms of sample illumination. The curves were normalized to an equal maximum fluorescence intensity, since this was somewhat higher in the resistant than in the susceptible chloroplasts.

portion of the fluorescence transients ($F_i - F_0$), expressed as a proportion of the total variable fluorescence ($F_m - F_0$), gave a higher value for the resistant biotype (0.41) than for the susceptible one (0.24). Similar values were obtained for triazine-resistant and susceptible biotypes of *Brassica campestris* L. [19]. The results of fluorescence induction measurements indicate that in resistant *E. canadensis* chloroplasts the midpoint oxidation-reduction potential of the Q_B/Q_B^- couple is decreased with respect to that of the Q_A/Q_A^- couple.

The conclusion drawn from fluorescence induction measurements was confirmed by thermoluminescence investigations of susceptible and resistant *E. canadensis* chloroplasts. In order to simplify the interpretation of the results of thermoluminescence measurements, special measuring conditions were chosen. Thermoluminescence was excited at -70°C, where the charging efficiency of the B_1 band [20] was very low and the main thermoluminescence band originating from charge recombination of the $S_2Q_B^-$ state [10,11], could be considered as a single band (B band). It was observed that in the glow curve of susceptible chloroplasts the B band was a narrow band, with peak temperature at around 32°C (Fig. 2). Surpris-

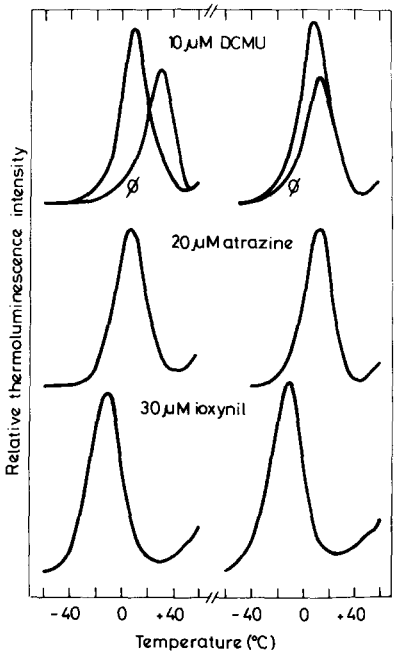

Fig. 2. Comparison of the effects of DCMU, atrazine and ioxynil on the thermoluminescence of chloroplasts isolated from triazine-susceptible (left side) and resistant biotype (right side) of *E. canadensis* L. The glow curves of untreated chloroplasts are labeled by ∅. Chloroplasts were suspended in a medium containing 0.4 M sorbitol/10 mM NaCl/1 mM $MnCl_2$/5 mM $MgCl_2$/2 mM EDTA/50 mM Hepes at pH 7.5 and incubated at room temperature in the presence of inhibitors for 5 min in the dark before thermoluminescence measurements. Thermoluminescence was excited by white light (10 $W \cdot m^{-2}$) for 30 s at −70°C. All samples contained 60% glycerol.

ingly, in the glow curve of resistant chloroplasts the B band was a little broadened and appeared at about 15°C. According to the theory of thermoluminescence [16], a shift in the emission temperature of the B band indicates that the midpoint potential of the Q_B/Q_B^- couple is lowered in the resistant chloroplasts relative to the sensitive ones.

After DCMU treatment of chloroplasts, the Q band (recombination of the $S_2Q_A^-$ state) appeared at about 10°C in the glow curves of both the susceptible and the resistant biotypes (Fig. 2), suggesting that in DCMU-treated chloroplasts the midpoint potentials of the S_2/S_3 and Q_A/Q_A^- redox couples are the same for both biotypes. However, the question arises whether the DCMU-treatment causes a change in the midpoint redox potentials of the S_2/S_3 and Q_A/Q_A^- couples. It has been reported that in the presence of DCMU or weak concentration of a reducing agent the thermoluminescence band which can be attributed to charge recombination of the $S_2Q_A^-$ state appears at the same temperature [10]. This result indicates that DCMU treatment does not influence the redox potentials of the S_2/S_3 and Q_A/Q_A^- couples. Consequently, our observation that in DCMU-treated chloroplasts the Q band appears at about the same temperature for both biotypes (Fig. 2) implies that the triazine resistance is also not accompanied by an alteration in the midpoint potentials of the S_2/S_3 and Q_A/Q_A^- redox couples. This result is in line with the conclusions of Vermaas et al. [21] who showed, by means of fluorescence decay measurements, that in the presence of diuron the rate of Q_A^- oxidation due to recombination with S_2 is very similar for triazine-susceptible and triazine-resistant *Brassica napus* thylakoids.

Fig. 2 also demonstrates that the amplitude of the Q band is apparently intensified relative to that of the B band. This observation can be explained by the two-electron gating mechanism of electron flow on the acceptor side of PS II. It has been reported that a small percentage of the secondary quinone acceptor pool remains in the semi-reduced state even in thoroughly dark-adapted chloroplasts [22]. After receiving a second electron, due to excitation of thermoluminescence, the doubly reduced secondary quinone, Q_B^{2-}, dissociates from its binding site within a few milliseconds, thereby decreasing the number of electron-transport chains which contribute to generation of the B band. On the other hand, at room temperature after DCMU addition, Q_A^- backreacts with the donor side of PS II within a few seconds [11,23]. Consequently, in dark-adapted chloroplasts after DCMU treatment, the primary quinone molecules Q_A are mainly in the oxidized state. Excitation of thermoluminescence induces charge separation ($S_2Q_A^-$) in the majority of centers. As a result, the amplitude of the Q band is enlarged in comparison with the amplitude of the B band corresponding to the original Q_B^-/Q_B ratio in dark-adapted chloroplasts (it varies with the dark-adaptation time of the chloroplasts and with the plant material used.). Our Hill reaction measurements demonstrated that thylakoid membranes of triazine-resistant *E.*

canadensis chloroplasts had a greatly reduced affinity for atrazine. In agreement with this, the B band was not replaced by the Q band in the glow curve of resistant chloroplasts even in the presence of 20 μM atrazine (Fig. 2). On the other hand, atrazine treatment of susceptible chloroplasts resulted in the appearance of the Q band, suggesting that the electron transport between Q_A and Q_B was interrupted by atrazine. The effects of the phenolic-type inhibitor ioxynil on the thermoluminescence were very similar in the two biotypes (Fig. 2). The Q band appeared at about $-10°C$ in the glow curves of both the sensitive and the resistant chloroplasts. The appearance of a thermoluminescence band at about $-10°C$ in the glow curve of ioxynil-treated chloroplasts may be explained by the existence of an additional site of action of phenolic-type inhibitors on the donor side of PS II {24,25]. This assumption is supported by the observation that chloroplasts having an inactivated water-splitting system exhibit a thermoluminescence band at about $-10°C$ [26].

According to Vermaas and Arntzen [4] the increased equilibrium concentration of Q_A^- in resistant chloroplasts as compared to susceptible ones can be explained either by a decreased affinity of Q_B to its binding site or by a decreased midpoint redox potential of the Q_B/Q_B^- couple with respect to that of the Q_A/Q_A^- couple. In thermoluminescence measurements a decrease in the affinity of plastoquinone for the quinone binding site would manifest itself as a decrease in the amplitude of the B band and a change in the midpoint redox potential of the Q_B/Q_B^- couple should appear as a shift in the peak position of this band. In our thermoluminescence measurements the amplitude of the B band was not considerably changed in the glow curve of triazine-resistant as compared to triazine-susceptible chloroplasts. On the other hand the peak position of the B band appeared at lower temperature in the glow curve of resistant chloroplasts than in that of sensitive chloroplasts (Fig. 2). The peak position of a thermoluminescence band is determined by the redox span between the positively charged donor and the negatively charged acceptor, which are interacting in the radiative charge recombination reaction resulting in thermoluminescence. The B band is assigned to the charge recombination of the $S_2Q_B^-$ redox state. Since according to our previous conclusion the midpoint redox potential of the S_2 state is not significantly altered in resistant chloroplasts, the shift in the peak position of the B band can be attributed solely to the lowering of the midpoint potential of the Q_B/Q_B^- redox couple. The theory of thermoluminescence provides an opportunity to calculate the value of the shift in the redox potential of the Q_B/Q_B^- couple. The glow curves of untreated and DCMU-treated chloroplasts were fitted by Randall-Wilkins theoretical curves, and the free energies of activation were determined for the Q and B bands according to the procedure given in Ref. 16. The results are listed in Table I. From a comparison of the free energies of activation obtained for the B band in the two biotypes, the extent of the shift in the midpoint potential of the Q_B/Q_B^- couple accompanying atrazine resistance is about 41 mV. Further, the redox distances between Q_A and Q_B in sensitive and resistant chloroplasts are 71 and 32 mV, respectively. In the knowledge of the redox distance between Q_A and Q_B, the equilibrium constant (K) for the equilibrium $Q_A^-Q_B \overset{K}{\rightleftharpoons} Q_AQ_B^-$ can be calculated [17]. In the following, the equilibrium constants for the sensitive and resistant chloroplasts will be designated K_S and K_R, respectively. The value of $K_S = 16$ obtained for the sensitive *E. canadensis* biotype is in good agreement with the values of 15–20 reported by Diner [27] for spinach, and estimated by Robinson and Crofts [23] for pea chloroplasts. According to Vermaas et al. [21], in pea thylakoids the apparent equilibrium constant of the equilibrium $Q_A^-Q_B/Q_AQ_B^-$ is about 10, whereas in triazine-susceptible *Brassica napus* thylakoids this equilibrium constant is a factor of about 2 higher.

Due to the lowered redox potential of Q_B, the equilibrium constant between Q_A and Q_B ($K_R = 3.5$) is much smaller in resistant *E. canadensis* chloroplasts than in susceptible ones. This means that for an electron shared between Q_A and Q_B the probability of the electron residing on Q_A at any given time is higher in the reaction centers of the resistant biotype. Under our experimental conditions (excitation of thermoluminescence at $-70°C$) there is only one 'free', electron in the Q_AQ_B complex, and thus the probability of finding Q_A in

the reduced form in the resistant chloroplasts is about 22% (in sensitive chloroplasts it is about 6%). The larger amount of reduced Q_A present in resistant chloroplasts before light absorption could limit the number of charge separations in PS II (closed reaction centers) and can account for the increased percentage of misses observed in O_2 yield measurements [7,8].

It must not be overlooked that the equilibrium constants for the two biotypes were determined at pH 7.5. It has already been pointed out by Lavergne [28] and recently by Crofts et al. [29] that the equilibrium constant of the equilibrium $Q_A^- \cdot Q_B/Q_A \cdot Q_B^-$ is pH-dependent, increasing as the pH is decreased. We have attempted to investigate the pH-dependence of the equilibrium constant by thermoluminescence measurements. It was observed that in the glow curve of DCMU-treated susceptible *E. canadensis* chloroplasts the peak temperature of the Q band (associated with the $S_2Q_A^-$ state) was practially independent of pH, indicating that neither Q_A nor the S_2 state is influenced considerably by pH change in the pH range 7.5–6.0 (Fig. 3). On the other hand, on change of the pH from 7.5 to 6.0, the peak position of the B band shifted from 32 to 45°C, a result which is in agreement with earlier observations [20,30]. Accepting that only the Q_B^- state and not the S_2 fraction that recombines with Q_B^- is dependent upon pH, the shift in the B band peak position corresponds to an increase of about 18 mV in the midpoint potential of the Q_B/Q_B^- pair, and consequently to a 2.8-fold increase in the value of the equilibrium constant ($K_S = 16$ at pH 7.5, and $K_S = 44$ at pH 6.0). This suggests that protonation of Q_B^- or of a neighbouring group [29] may play an important role in stabilizing Q_B^-, causing a decrease in the amount of $Q_A^- \cdot Q_B$ relative to $Q_A \cdot Q_B^-$ (H^+) with decreasing pH. A similar suggestion has been made by Vermaas et al. [21] on the basis of fluorescence decay and S_2 decay measurements. In contrast with this, according to Ref. 30, the shift of the B band can be solely interpreted by the effect of pH on the S states of the water-splitting system. However, this interpretation can hardly explain why the $S_2Q_B^-$ state (B band) behaves differently from the $S_2Q_A^-$ state (Q band) under the effect of low pH. In the resistant chloroplasts, investigation of the pH effect on the

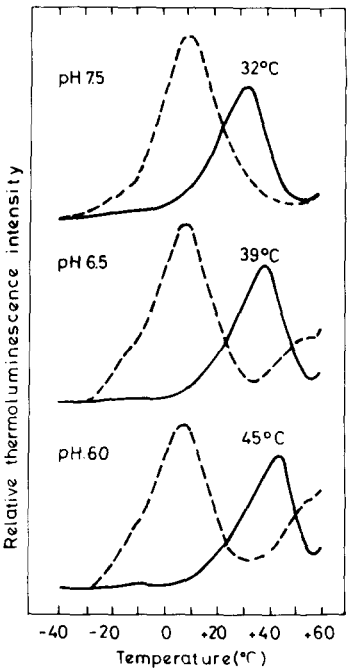

Fig. 3. Effect of pH on the thermoluminescence of isolated susceptible chloroplasts of *E. canadensis* in the presence (— — —) and absence (———) of 10 μM DCMU. Chloroplasts were suspended in the standard suspension medium, except that the 50 mM Hepes was replaced by 50 mM phosphate buffer of various pH values. All samples contained 30% glycerol.

semiquinone equilibrium between Q_A and Q_B encountered difficulties. Decrease of the pH of the medium resulted in the gradual appearance of the B_1 band, even on low-temperature excitation of thermoluminescence, and the glow curve culd no longer be fitted well with a single-component band. In order to demonstrate the appearance of the B_1 band upon lowering the pH of the medium, the effect of pH on the thermoluminescence of resistant chloroplasts is shown in the pH range 7.5–5.0 (Fig. 4). The appearance of the B_1 band in the glow curve of resistant chloroplasts is in correlation with the observation that in resistant thylakoids there is a very stable, slowly decaying fraction of the S_2 state which causes an abnormally high O_2 evolution at the second flash [5,6,21]. At −70°C, where thermoluminescence is excited, electron transport is blocked between Q_A and Q_B and only one electron can be transferred from the donor side of PS II to the acceptor side. Conse-

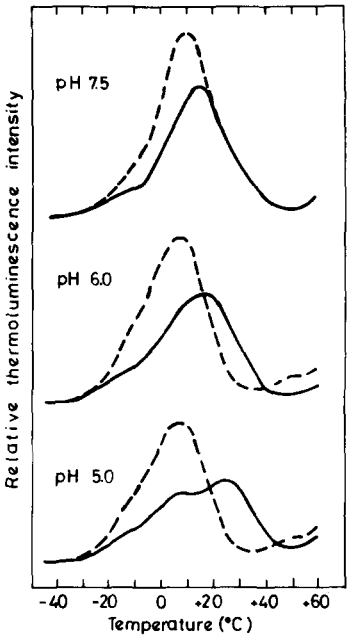

Fig. 4. Effect of pH on the thermoluminescence of isolated resistant chloroplasts of *E. canadensis* in the presence (— — —) and absence (———) of 10 μM DCMU. Measuring conditions as in Fig. 3.

quently, in a fraction of electron-transport chains, which contain the water-splitting system in S_2 state, the $S_2 \rightarrow S_3$ transition can proceed upon illumination of chloroplasts. As a result the B_1 band, which is associated with the S_3 state [20], appears in the glow curve of resistant chloroplasts. The presence of a hidden B_1 band under the envelope of the B band in the glow curve of resistant chloroplasts at pH 7.5 can cause an error in the estimation of the equilibrium constant, K. Additional error may be introduced into the value of K when using the Randall-Wilkins equation in the curve-fitting procedures [17]. Due to these distortions the extent of the shift in the midpoint potential of the Q_B/Q_B^- couple is probably underestimated in our calculations and the value of K obtained for resistant *E. canadensis* chloroplasts ($K_R = 3.5$) is higher than those published in the literature. The value of K was estimated to be about 1 in *B. napus* thylakoids [5]. Similar value for K can be calculated from the data presented in [9] for triazine resistant *A. hybridus*. The fast initial fluorescence rise in resistant *E. canadensis* chloroplasts to about 41% of the total variable fluorescence (Fig. 1) also indicates a K value of less than 1.5 rather than 3.5 calculated from thermoluminescence measurements. In conclusion, we can say that the data presented in Table I for sensitive chloroplasts can be considered as real values, however, for resistant chloroplasts they are only approximations of the actual values.

The question arises as to whether the alteration in the midpoint redox potential of Q_B is a general trait of triazine resistance, and whether it is always displayed as a shift in the peak position of the B thermoluminescence band. To answer this question, the thermoluminescence of triazine-sensitive and resistant biotypes of *Amaranthus retroflexus, Solanum nigrum* and *Nicotiana plumbaginifolia* were compared (Fig. 5). It was observed that the peak position of the B band was shifted to lower temperatures in the glow curves of the resistant biotypes of all three plant species. Moreover, the extent of the shift varied with the plant species. These results, together with those of previous publications [4,5,7,9] indicate, that the triazine-resis-

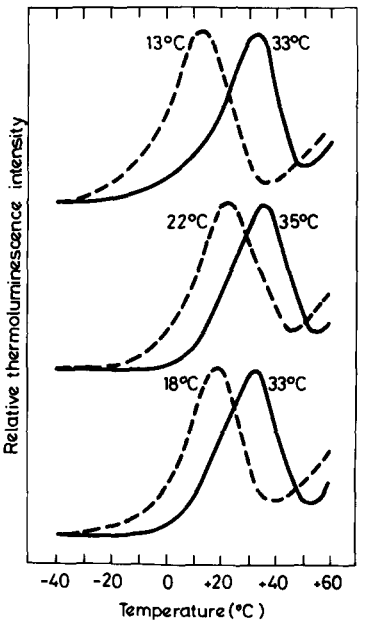

Fig. 5. Thermoluminescence of chloroplasts isolated from triazine-susceptible (———) and resistant (— — —) biotypes of *A. retroflexus* (upper curves), *S. nigrum* (middle curves) and *N. plumbaginifolia* (bottom curves). Thermoluminescence was excited at −70°C. All samples contained 30% glycerol.

tance trait is accompanied by a decrease in the midpoint oxidation-reduction potential of the secondary quinone acceptor of PS II. In addition it can be concluded that the extent of the shift in the redox potential of Q_B is dependent on the plant species. It has been shown earlier that thermoluminescence measurements can provide valuable information about the sites and modes of action of PS II herbicides [31,32]. The present study demonstrates that the thermoluminescence method can also be applied in the investigation of the altered electron-transport properties of triazine-resistant plants. Since small changes in the midpoint redox potential of Q_B are reflected as large shifts in the peak position of the B band, we suggest the thermoluminescence technique as a sensitive and alternative assay, besides fluorescence transient analysis [18,19,33], for the fast determination of triazine resistance in plants.

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