Inhibition of the Photosynthetic Electron Transport by Pyrethroid Insecticides in Cell Cultures and Thylakoid Suspensions from Higher Plants

Klaus P. Bader and Judith Schüler

Universität Bielefeld, Fakultät für Biologie, Lehrstuhl für Zellphysiologie, Postfach 10 01 31,
D-33501 Bielefeld, Bundesrepublik Deutschland

Dedicated to Professor Horst Senger on the occasion of his 65th birthday

Z. Naturforsch. **51 c,** 721–728 (1996); received April 25/June 20, 1996

Photosynthesis, Electron Transport, Oxygen Evolution, Pyrethroid Insecticide, Cell Culture

Synthetic pyrethroid insecticides with different molecular structures have been investigated with respect to their effect on photosynthetic electron transport reactions in chloroplast suspensions and cell cultures from higher plants. The fluorescence induction curves of tobacco (*Nicotiana tabacum*) leaves and tomato cells were substantially affected by permethrin and cypermethrin resulting in a strong increase of the maximum fluorescence. Application of different concentrations (0.3–1.2 mM) of the respective chemical abolishes virtually any kinetics of the normal Kautsky effect. Oxygen evolution from cell cultures from tomato (*Lycopersicon peruvianum*) was completely inhibited by cypermethrin. Analysis of partial reactions of the photosynthetic electron transport showed that both a methylviologen-mediated Mehler reaction and a ferricyanide-driven Hill reaction were quantitatively inhibited by *e.g.* fenvalerate. On the other hand, neither a silicomolybdate-driven Hill reaction nor a methylviologen-driven Mehler reaction using dichlorophenol indophenol/ascorbate as electron donors could be inhibited by the pyrethroid. The analyses suggest that pyrethroid insecticides interfere with the photosynthetic electron transport at the same site as urea-type herbicides do. Depending on the molecular structure and on the halogen compound in the molecule, however, different pyrethroids are more or less phytotoxic to the investigated photosynthetic membranes – cypermethrin with two Cl-substituents requires much higher concentrations to be applied for significant inhibition of the electron transport reactions than the Br-derivative deltamethrin does. Moreover, qualitative differences have to be taken into account. In the case of fenvalerate the effect seems to exist in a type of all-or-nothing reaction when the reaction centres are inhibited by the pyrethroid. None of the S-states nor the transition probabilities are specifically influenced by increasing concentrations of fenvalerate. In the case of deltamethrin, however, it was found that the overreduced state S_{-1} is significantly increased at the expense of both S_1 and S_0. Moreover, the miss parameter α is increased in the case of deltamethrin addition. The results and the significance of different substituents for the investigated pyrethroids are discussed.

Introduction

Since years, herbicides are well-investigated with respect to their effect on photosynthetic electron transport reactions; dichlorophenyl dimethylurea (DCMU) as inhibiting herbicide has become the classical testing agent for a photosystem II reaction (for a review see Renger, 1986). Detailed analyses have been carried out investigating the inhibition of electron transport reactions in isolated cytochrome complexes, photosynthetic bacteria, cyanobacteria and higher plants (Oettmeier *et al.*, 1991; Trebst *et al.*, 1993; Oettmeier *et al.*, 1994). Much work has also been done dealing with herbicides and the inhibition of carotenoid- and/or chlorophyll biosynthesis. Many of the so-called 'bleaching herbicides' have been shown to act on phytoene desaturase as one of the relevant enzymes introducing double bonds in the precursors of β-carotene (Böger and Sandmann, 1993; Sandmann *et al.*, 1991). The correlation between the inhibitory effect of the carotenoid biosynthesis inhibitor norflurazon, illumination and D1 degradation has been described (Sandmann *et al.*, 1993) as well as the mechanism of action of peroxidizing herbicides (Böger and Wakabayashi, 1995). How-

---

Abbreviations: asc, ascorbate; DCMU, 3-(3,4-dichlorophenyl)-N-N'-dimethylurea; DCPIP, dichlorophenol indophenol; LP 396, *Lycopersicon peruvianum* culture 396; MV, methylviologen; OEC, oxygen evolving complex; Y, flash induced oxygen evolution amplitude.

Reprint requests to Prof. K. P. Bader.
Fax: 0521/1065626.

0939–5075/96/0900–0721 $ 06.00 D

ever, in the general field of plant protecting agents, only herbicides have been thoroughly investigated concerning their interaction and interference with the photosynthetic electron transport chain. From such work it is known that herbicides like the above mentioned DCMU interact with Q_B on the acceptor side of PS II. (For a review see Oettmeier, 1992.) Moreover, substituted phenols have been shown to orientate differently in the Q_B-site in comparison to the urea-type herbicides. Mutation in the D1 protein lead to substantial tolerance of *Chlamydomonas* towards phenolic inhibitors (Trebst *et al.*, 1993). Details on the quinone binding sites on cytochrome complexes both in the photosynthetic and respiratory pathway including the use of site-specific inhibitors for mutagenesis of the respective binding site has been reviewed by Frank and Trebst (1995).

Only scattered reports, however, deal with the effects of other plant-protecting substances like *e.g.* insecticides on plants (Bhunia *et al.*, 1993; Mishra *et al.*, 1992) what can easily be explained by the fact that insecticides (fungicides or others) are supposed to have a negative effect only on their specific target organism but should have rather good compatibility and tolerance with respect to the treated plants. From this point of view synthetic pyrethroid insecticides which can be derived from the naturally occurring (*Chrysanthemum*) pyrethrins might be of specific interest. Analysis of these substances in connection with photosynthesis might be not only relevant for physiological studies but should also allow new insights into structural necessities of modern and ecologically acceptable plant protection chemicals.

Materials and Methods

Cell cultures from tomato (*Lycopersicon peruvianum* LP 396) were kindly supplied by Prof. Dr. H.-P. Mühlbach, Institut für Allgemeine Botanik in Hamburg, Germany and cultivated in two-tire vessels containing the cells in culture medium and (separately) a 2M $K_2CO_3/KHCO_3$-buffer which provided 2% CO_2 in the gas atmosphere. Details of the procedure have been described earlier (Stöcker *et al.*, 1993; Hüsemann and Barz, 1977).

Chloroplasts from tobacco (*Nicotiana tabacum* var. John William's Broadleaf) were isolated by mechanical homogenization of leaflets, centrifugation and suspension in buffer containing high concentrations of sucrose. Prior to the experiments, aliquots from the chloroplast suspension were diluted in reaction buffer (Tricine 0.03 M / KCl 0.06 M – pH 7.5) (Homann and Schmid, 1967). Some experiments have been carried out with intact leaves or leaflets also from this tobacco strain.

Fluorescence measurements were performed in a laboratory-built fluorometer. 1 ml reaction assay contained about 50–100 µg of chlorophyll in suspension buffer, reaction buffer (*vide supra*) and additions as indicated in a glass cuvette exposing a surface of approx 16 cm^2 to the light source. The light source illuminated the sample via an electrically triggered shutter, a blue plexiglas filter and a SCHOTT BG 28 filter. Fluorescence was detected by an EMI photomultiplier Type 9658R under a high tension of 1000–1100 V. Fluorescence induction curves were recorded on a 5115 storage oscilloscope from Tektronix, equipped with a 5A22N differential amplifier and a 5B12N dual time base.

Electron transport reactions were carried out in a Clark-type electrode from Rank Bros., Bottisham, Great Britain. Oxygen evolution from a silicomolybdate- or a ferricyanide-mediated Hill reaction, or oxygen uptake from a water → anthraquinone-2-sulfonate- or a dichlorphenolindophenol → anthraquinone-2-sulfonate Mehler reaction were measured and recorded on a SE 130 Recorder from BBC-Metrawatt. The assays were illuminated with a Leitz projector (Prado Universal) through a red plexiglas filter ($580 \text{ nm} < \lambda < 750 \text{ nm}$).

Flash-induced oxygen evolution was detected on the three-electrode-system developed by Schmid and Thibault (1979). The signals were processed by means of an Atari Mega ST 4 computer (Schulder *et al.*, 1992). Subsequently, the experimental data were fitted using the VOYONS general modelling from Thiéry (1991) on an IBM compatible computer. Details have been described (Thibault and Thiéry, 1981; Thibault, 1982).

Pyrethroids were diluted in water and applied in the concentrations indicated in the figures and in the figure legends. Cypermethrin (Ripcord 10) was obtained from C.F. Spiess & Sohn, Kleinkarlbach, fenvalerate (Sumicidin 10) from Amer. Cyanamide, Ingelheim, deltamethrin (Decis EC 2,5) from Hoechst, Frankfurt and permethrin (Ambush) from Deutsche ICI, Frankfurt.

Results

Leaves from higher plants show the typical fluorescence emission curves well-known from the literature and described as Kautsky effect during the initial phases of the illumination period. According to the current concept the fluorescence originates almost exclusively from photosystem II (at room temperature) and reflects the redox state on the acceptor side of PS II, as the first quinone acceptor, Q_A, in its oxidized state acts as a fluorescence quencher (Duysens and Sweers, 1963). The fluorescence can, however, be substantially influenced and affected by pretreatment of the leaf sections with the pyrethroids permethrin and cypermethrin (Fig. 1). (Such synthetic pyrethroids are often used in the field of plant protection as effective insecticides. Many of these chemicals have been derived from '*Pyrethrum*' a mixture of the physiologically occurring pyrethrins from *Chrysanthemum* species.) Fig. 1a shows that the applied pyrethroids increase the fluorescence yield of leaf sections from tobacco (*Nicotiana tabacum* var. John William's Broadleaf). This observation suggests that synthetic pyrethroid insecticides interact with the photosynthetic electron transport chain and influence the redox conditions of the primary electron acceptor of photosystem II in the *in vivo* conditions of intact leaves. Furthermore, it can be concluded that the interaction can be observed with various pyrethroids with different molecular structures. Permethrin increases fluorescence at a lower concentration to a higher extent than cypermethrin does. It should be noted that the only difference in the molecular structure between the two pyrethroids consists of the lack of the CN-group in the case of permethrin (Fig. 2). The effect of pyrethroids on fluorescence was analyzed in detail with cell cultures from tomato (*Lycopersicon peruvianum*). These cell suspensions show virtu-

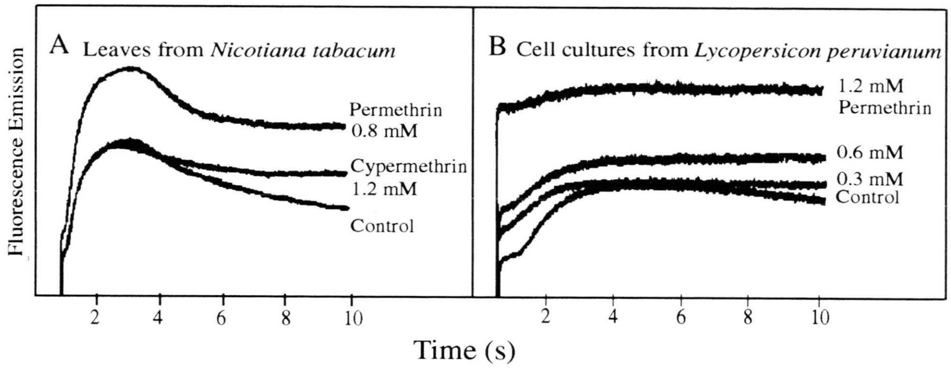

Fig. 1. Effect of synthetic pyrethroids on the fluorescence induction curves from tobacco leaves (*Nicotiana tabacum*) (A) and cell cultures from *Lycopersicon peruvianum* (B). Plants had been sprayed (completely wetted) with the pyrethroids 1 day before the measurements. Leaves had been cut from the plants and dark incubated 10 min before illumination (A). Cell suspensions were supplemented with the pyrethroid and dark adapted 5 min before the measurements (B).

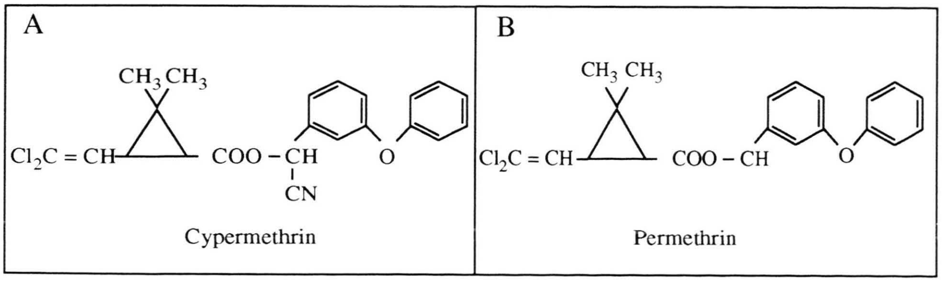

Fig. 2. Molecular structure of the synthetic pyrethroids cypermethrin (A) and permethrin (B).

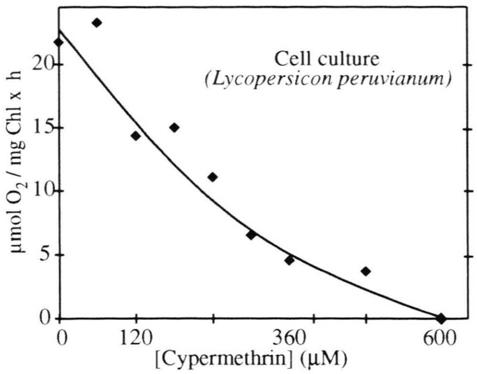

Fig. 3. Effect of increasing concentrations of cypermethrin on the oxygen evolution capacity in cell cultures from *Lycopersicon peruvianum*. Photosynthetic oxygen evolution was detected without artificial electron acceptor.

ally the same (principal) fluorescence induction curves and are, on the other hand, much more accessible for exogenously added chemicals than whole leaves are. Addition of the pyrethroid permethrin strongly increases the fluorescence yield of the cell cultures and nearly abolishes any kinetics of the normal Kautsky effect (Fig. 1b). This hints at an inhibition of the electron transport in the region between photosystems II and I. The same holds true for assays with chloroplast suspensions from tobacco (results not shown) although in most cases the kinetics of the fluorescence emission curves are less pronounced with these preparations due to a mechanical disrupture of parts of the electron transport chain (membrane structure) during the isolation procedure.

Cell cultures from tomato are capable of performing photosynthetic electron transport using water as physiological electron donor at rates of about 40–50 µmol O_2 x mg Chl^{-1} x h^{-1}. Consequently, an inhibition of the electron transport should result in an inhibition of the water oxidation capacity of the cells. Fig. 3 shows that this is indeed the case. Concentrations at about 600 µM of the pyrethroid cypermethrin reduces oxygen evolution of the cell cultures to zero. This means that the insecticide is capable of completely blocking the photosynthetic electron transport chain in higher plants. Following this observation, the question arises, wether the effect is based on a general and unspecific inhibition of the electron transport as a whole. Another possibility would be that the chemical exerts its effect in a specific region or even at a specific site which can be localized by analyzing specific and distinct parts of the electron transport chain. In order to clarify this question we measured the oxygen gas exchange of tobacco chloroplasts in water → methylviologen, water → ferricyanide, water → silicomolybdate and dichlorophenolindophenol/ascorbate → methylviologen partial reactions of the electron transport. For this and for the following experiments we used two other pyrethroids, namely fenvalerate and deltamethrin (Fig. 4). These substances have been chosen because of the (possibly) relevant structural differences of the molecules. In the case of deltamethrin the two chlorides on the dichlorovinyl-dimethylcyclopropyl side of the molecule are changed to two bromides. Fenvalerate shows a completely different structure in this part of the molecule. It contains an isobutyl component and a chlorophenyl ring instead. Fig. 5 clearly shows that these pyrethroids have the same inhibitory effect on the electron transport as permethrin and cypermethrin have as far as the overall effect on the whole chain is concerned. This effect must be

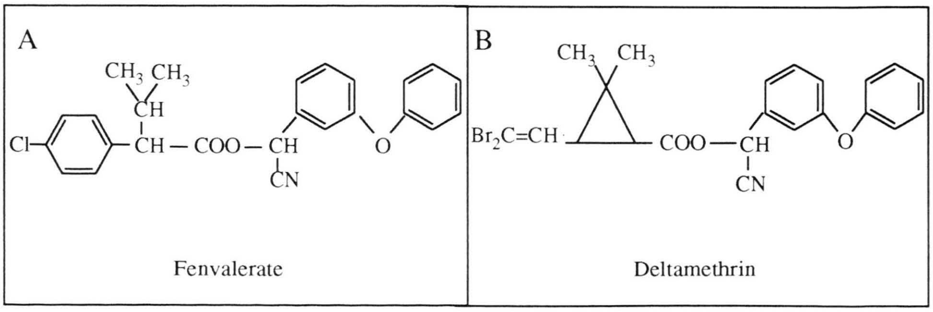

Fig. 4. Molecular structure of the synthetic pyrethroids fenvalerate (A) and deltamethrin (B).

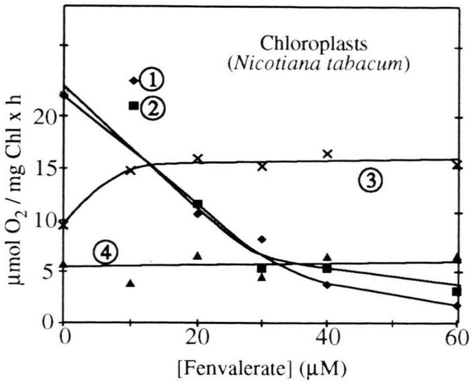

Fig. 5. Effect of increasing concentrations of fenvalerate on partial reactions of the photosynthetic electron transport in thylakoids from *Nicotiana tabacum*. **(1)** $H_2O \rightarrow$ FeCy; **(2)** $H_2O \rightarrow$ MV; **(3)** PS I-DCPIP/asc → MV; **(4)** $H_2O \rightarrow$ SM. For comparison positive values of O_2-evolution (**1** + **4**) and negative values of O_2-uptake (**2** + **3**) are depicted in the same coordinate system.

located on the acceptor side of photosystem II. Electron transport running through both photosystems (water → MV) is completely inhibited what is also the case for a ferricyanide-mediated Hill reaction. A silicomolybdate Hill reaction, however, remains completely unaffected even by the highest concentration of the applied pyrethroids. A DCPIP/asc → MV Mehler reaction was also not inhibited; in this case even a slight stimulation was observed which appeared at relatively low concentrations and remained constant over the concentration range which we analyzed. (This stimulatory effect i.e. an interaction of the pyrethroids with photosystem I shall only be mentioned here and will be analyzed in a forthcoming paper.) From our results it can be concluded that the site of inhibition of pyrethroids can be localized on the acceptor side of photosystem II between the sites where silicomolybdate and ferricyanide accept electrons from the transport chain. Thus, it appears that these insecticides interact with the photosynthetic electron transport chain exactly at the same site as herbicides (*e.g.* diuron) do.

In further experiments we analyzed the effect of pyrethroids on the flash induced oxygen evolution in the frame of the coherent Kok model. All oxygen amplitudes under a series of short (5 μs) saturating flashes are inhibited by different concentrations of deltamethrin, cypermethrin (Fig. 6) and other pyrethroids. The highest sensitivity of photosynthetic membranes to the pyrethroids was observed with deltamethrin; a concentration of 5 μM completely inhibited the flash induced oxygen evolution, whereas in the case of fenvalerate about 5–10-fold higher concentrations were required (Fig. 6a,b). Furthermore, the kinetics of the concentration dependence appear to be different in the two cases and a specific effect of deltamethrin on individual *S*-states has to be regarded. In the case of fenvalerate the ratio Y_{max}/Y_{min} remains constant over a wider concentration range of the pyrethroid than it was observed with deltamethrin. This might be explained by the interpretation that the chemical inhibits all *S* states to about the same extent. For further interpretation of Y_{max}/Y_{min} see

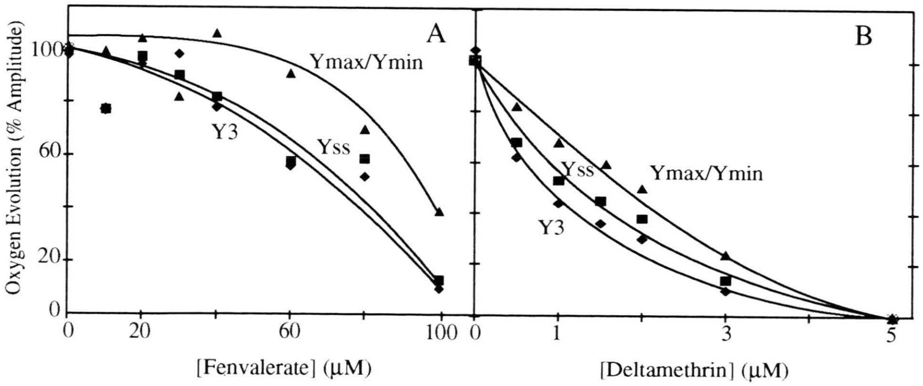

Fig. 6. Effect of synthetic pyrethroids on the flash induced oxygen evolution in tobacco chloroplasts under the third flash of a train of short (5 μs) saturating light flashes (Y_3), the steady state oxygen evolution amplitude (Y_{ss}) and on the ratio maximum amplitude/minimum amplitude (Y_{max}/Y_{min}). (A) Fenvalerate; (B) Deltamethrin.

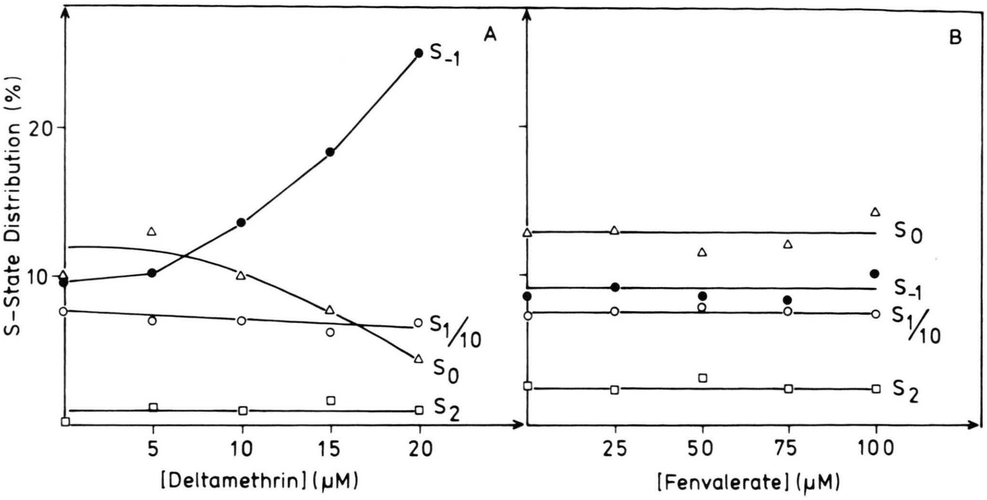

Fig. 7. Effect of deltamethrin (A) and fenvalerate (B) on the S-state distribution in tobacco chloroplasts calculated on the basis of a 5-state Kok model.

also Bader and Höper, 1994. In the case of deltamethrin, however, Y_{max}/Y_{min} is decreased already in the early phases of inhibition. As Y_{max} in this case means Y_3, one might assume that S_1 is preferentially hit by the pyrethroid. In order to substantiate this idea we fitted the oxygen evolution sequences by means of the VOYONS mathematical program developed by Thiéry (1991). Calculation of the *S*-state distribution yielded values of 10–15% S_0, 83–88% S_1, approx. 1 % S_2 and approx. 1% S_3 according to the 4-state Kok model for the controls. Fit of the oxygen amplitudes using the 5-state Kok model resulted in a value of approx. 10% for S_{-1} at the expense of S_1. Fig. 7 shows the dependence of the *S*-state distribution in the presence of increasing concentrations of deltamethrin and fenvalerate in the 5-state Kok model. From this fit it can be concluded that in fact S_1 but even more S_0 appear to be decreased by deltamethrin. The effect seems to consist of a (chemical) reduc-

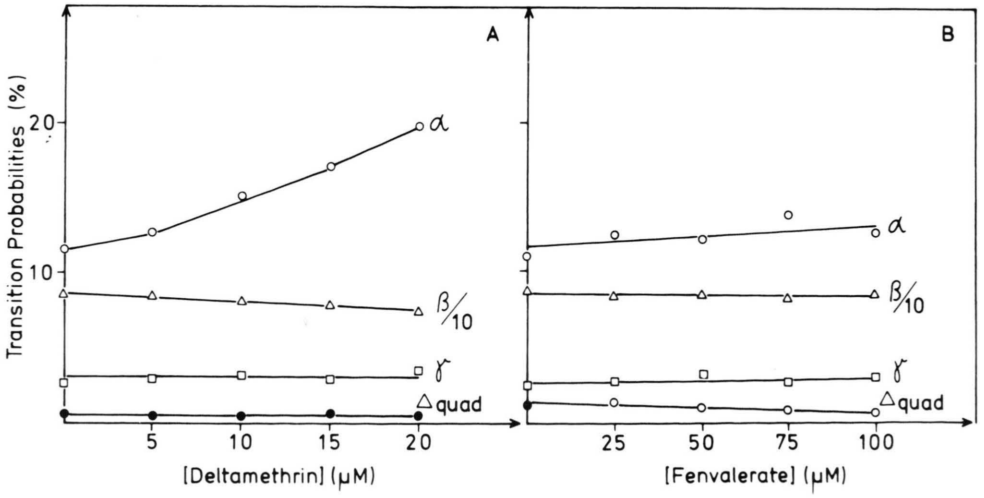

Fig. 8. Effect of deltamethrin (A) and fenvalerate (B) on the transition probabilities misses (α), successes (β) and double hits (γ) and on the quadratic deviations (Δ quad) of the fit.

tion of the reaction centres; this can also be derived from Fig. 7 showing that the 'overreduced' state S_{-1} increased by a factor of about 2.5. Moreover, Fig. 8 shows that the transition probabilities are attached. The miss parameter α was substantially increased upon deltamethrin addition with the concomitant decrease of successful transitions β. The double hit parameter γ seems not to be influenced by the pyrethroid. The results show that insecticides like pyrethroids can interact very specifically not only with photosynthetic membranes as a whole but with the *S*-states of the OEC in particular. Redox states are converted from S_1 to S_{-1} and the functioning of the complex as evidenced by the transition probabilities is substantially influenced.

The described effects are largely specific for deltamethrin (or related molecular structures) as similar observations cannot be made (or to a much smaller extent) following the addition of fenvalerate. In Fig. 6 it was shown that the ratio Y_{max}/Y_{min} remained constant over a relatively wide range of the pyrethroid concentration dependency. In this case, the inhibitory effect of the insecticides seems to consist of a more general and unspecific impairment of the OEC. Figs. 7 and 8 show part of the results of the mathematical fit for fenvalerate. It is clearly seen that neither the *S*-state distribution nor the transition probabilities are substantially influenced by this pyrethroid (Figs. 7b/8b). (Only a slight increase of the miss parameter α can be discussed.) This means that fenvalerate acts on the reaction centres in a type of all-or-nothing reaction whereas the OECs in the case of deltamethrin are attached in their specific redox and transition conditions before/when the function is blocked.

Discussion

Synthetic pyrethroids have been derived from the physiologically occuring pyrethrins and are often used as insecticides in the frame of modern plant protection. However, the present paper shows that first a strong impact of certain pyrethroids on photosynthetic electron transport reactions has to be considered and that second large differences exist between the effects of different pyrethroids depending on details of the chemical structure of the respective molecule. In this context it should be mentioned that structure-activity correlations have been described for fluridone derivatives in the case of phytoene desaturase. The significance of the methyl group as N-substituent and the lower indispensability of the keto group, the aromatic nature of the cyclic structure and other parameters have been investigated (Sandmann *et al.*, 1992). From the detailed studies of partial reactions of the electron transport in chloroplasts we conclude that the investigated insecticides have a binding site and show interaction with the photosynthetic electron transport chain at the same site where several (the urea-type) herbicides interfere, namely Q_B. Like the herbicide DCMU, the investigated pyrethroids strongly inhibit PS II reactions like the ferricyanide-driven Hill reaction and the Mehler reaction $H_2O \rightarrow MV$. Neither a silicomolybdate-driven Hill reaction nor a PS I reaction are inhibited. Comparison of the effects of different pyrethroids yield further insights into the relevant structural components of the molecules which are responsible for a specific interaction with photosystem II.

As the sensitivity of the examined photosynthetic membranes to the pyrethroids permethrin, cypermethrin, fenvalerate and deltamethrin differed by one to two orders of magnitude, the two-carbocycles-side of the molecules can not be essential for the interactions. This side of the molecules is identical for all of the tested pyrethroids (apart from the ether bridge between the carbocycles in the case of deltamethrin). Thus, the halogen side of the molecules must be responsible for the observed phytotoxicity *i.e.* the specific interaction of the insecticides with the D1 protein. Consequently, the inhibitory effect of herbicides like DCMU must also be based on the dichlorophenyl-side of the molecule and can, possibly, be increased by exchanging the chloride to other halogens. This might be derived from the experiments showing that deltamethrin with two bromides inhibits photosynthesis to a higher extent than fenvalerate with a chlorophenyl or permethrin with its dichlorovinyl group does. Like the other structural components of the investigated pyrethroids also the cyclopropyl part of the molecule can not be responsible for the interaction with Q_B, as fenvalerate with a relatively good effect lacks this structure. Thus, it can be concluded from the results that the inhibitory effect of the measured pyrethroids increases in the sequence cypermethrin < permethrin < fenvalerate < deltamethrin and that the two bromides are the structural components responsible for the strong effect in the case of deltamethrin.

Acknowledgement

The authors thank Prof. Dr. H.-P. Mühlbach, Arbeitsbereich Genetik, Universität Hamburg for the cell cultures from tomato (*Lycopersicon peruvianum*) and for help with the cultivation of the cells.

Bader K.P. and Höper S. (1994), Stimulatory effects of an ammonium salt biocide on photosynthetic electron transport reactions. Z. Naturforsch. **49c**, 87–94.

Bhunia A.K., Roy D. and Banerjee S.K. (1993), Carbaryl-induced effects on glutathione content, glutathione reductase and superoxide dismutase activity of the cyanobacterium *Nostac muscorum*. Lett. in Appl. Microbiol. **16**, 10–13.

Böger P. and Sandmann G. (1993), Pigment biosynthesis and herbicide interactions. Photosynthetica **28**, 481–493.

Böger P. and Wakabayashi K. (1995), Peroxidizing herbicides: I. Mechanism of action. Z. Naturforsch. **50c** , 159–166.

Duysens L.N.M. and Sweers H.E. (1963), Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In: Studies on Microalgae and Photosynthetic Bacteria. (Special Issue of: Plant Cell Physiol.) **17**, 353–372.

Frank K. and Trebst A. (1994), Quinone binding sites on cytrochrome *b/c* complexes. Photochem. Photobiol. **61**, 2–9.

Homann P. and Schmid G.H. (1967), Photosynthetic reactions of chloroplasts with unusual structures. Plant Physiol. **42**, 1619–1632.

Hüsemann W. and Barz W. (1977), Photoautrophic growth and photosynthesis cell suspension cultures of *Chenopodium rubum*. Physiol. Plant. **40**, 77–81.

Mishra D.P., Singh R.L. and Gupta R.K. (1992), Pesticide induced response in maize chloroplast lipids. Photosynthetica **26**, 441–444.

Oettmeier W. (1992). Herbicides of photosystem II. In: Topics in Photosynthesis, Vol. **11**. Elsevier, Amsterdam, 349–408.

Oettmeier W., Hilp U., Draber W., Fedtke C. and Schmidt R.R. (1991), Structure-activity relationships of triazinone herbicides on resistant weeds and resistant *Chlamydomonas reinhardtii*. Pestic. Sci. **33**, 399–410.

Oettmeier W., Masson K. and Soll M. (1994), Inhibition of electron transport through the Q-p site in cytochrome *b/c*-1 complexes by acridones. Biochim. Biophys. Acta **1188**, 125–130.

Renger G. (1986), Herbicide interactions with photosystem II: Recent developments. Physiol. Vég. **24**, 509–521.

Sandmann G., Kowalczyk G., Schroeder S., Taylor H.M. and Böger P. (1992), Quantitative structure-activity relationship of fluridone derivates with phytoen desaturase. Pestic. Biochem. Physiol. **42**, 1–6.

Sandmann G., Kuhn M. and Böger P. (1993), Carotenoids in photosynthesis: Protection of D1 degradation in the light. Photosynth. Res. **35**, 185–190.

Sandmann G., Schmidt A., Linden H. and Böger P. (1991), Phytoene desaturase, the essential target for bleaching herbicides. Weed Sci. **39**, 474–479.

Schmid G.H. and Thibault P. (1979), Evidence for a rapid oxygen-uptake in tobacco chloroplasts. Z. Naturforsch. **34c**, 414–418.

Schulder R., Burda K., Strzalka K., Bader K.P. and Schmid G.H. (1992), Study on the parameters affecting oxygen release time measurements by amperometry. Z. Naturforsch. **47c**, 465–473.

Stöcker S., Guitton M.-C., Barth A. and Mühlbach H.-P. (1993), Photosynthetically active suspension cultures of potato spindle tuber viroid infected tomato cells as tools for studying viroid: Host cell interaction. Plant Cell Reports **12**, 597–602.

Thibault P. (1982), Contribution à l'étude des propriétés de l'émission photosynthétique d'oxygène. Recherche d'un modèle cohérent. Thèse Doct. d'Etat, Univ. Aix-Marseille II.

Thibault P. and Thiéry J.M. (1981), 1er Séminaire Ecole de Biologie Théorique, Paris pp. 283–297 (Ensta ed. Paris).

Thiéry J.M. (1991), „Voyons" Programme de Simulations Conversationnelles en Physico-Chimie et en Agronomie, in: Logiciels pour la Chimie, ISBN 2–903532–05–2, pp 292–293 (Antonot, N., Come, G.M., Gartiser, T., Guidon, J. and Soulié, E., eds.) Soc. Fr. Chimie (Paris) et Agence Nat. Logiciel (CNRS, Nancy).

Trebst A., Hilp U. and Draber W. (1993), Response in the inhibitor efficiency of substituted phenols on PS II activity in six mutants of the D1 protein subunit in *Chlamydomonas reinhardtii*. Phytochemistry **33**, 969–977.