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DIFFERENTIAL EFFICIENCY OF PHOTOSYNTHETIC OXYGEN EVOLUTION IN FLASHING LIGHT IN TRIAZINE-RESISTANT AND TRIAZINE-SUSCEPTIBLE BIOTYPES OF *SENECIO VULGARIS* L.

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Photosynthetic oxygen evolution in response to flashing light was studied in triazine-susceptible and triazine-resistant biotypes of *Senecio vulgaris* L. Studies were conducted to determine if the modification of the herbicide-binding site which confers *s*-triazine resistance also affects the oxygen-evolving system. Oxygen evolution was measured using a Joliot-type oxygen-specific electrode on broken, stroma-free chloroplasts of both biotypes. We observed abnormal patterns of oxygen evolution in resistant chloroplasts. The $S_1' \rightarrow S_2$ transition is slower while the S_2 decay is faster. The $S_2' \rightarrow S_3$ transition, in contrast, is slightly faster in resistant chloroplasts, while the decay of the S_3 state is the same as in susceptible chloroplasts. These altered kinetics may be due to altered $Q \rightarrow B$ (B^-) electron flow in resistant chloroplasts. These results are also consistent with the hypothesis that back-reactions from the reducing (acceptor) side of Photosystem II to the oxidizing (donor) side occur with greater frequency in resistant than susceptible chloroplasts. These events are responsible for lower oxygen yield and increased 'misses' and 'double hits,' resulting in abnormal yield patterns and lower quantum yield of CO_2 fixation in resistant chloroplasts compared to the susceptible ones.

Introduction

In recent years, biotypes of several weed species resistant to the photosynthetic inhibitors, the *s*-triazine herbicides, have been reported [1,2]. One species, *Senecio vulgaris* L., common groundsel, has been the basis of extensive studies since the discovery of the resistant biotype in 1970 [3,4]. It is now generally believed that in *s*-triazine-resistant biotypes, a chloroplast membrane alteration at the herbicide-binding site at PS II has resulted in the loss of *s*-triazine binding, and therefore, lack of inhibition of the light reactions of photosynthesis [5–7]. This alteration in the PS II complex is accompanied by changes in the rate of photosynthetic electron transport and O_2 evolution in chloroplasts of triazine-resistant *S. vulgaris* biotypes [7–9]. A reduction of photosynthetic efficiency in resistant chloroplasts has resulted in a lowered capacity for net carbon fixation and lower quantum yields measured in whole leaves of the resistant biotype, making it much less fit, when compared to the susceptible biotype [8].

Studies of O_2 evolution as a means of understanding photosynthetic efficiency at the chloroplast level demonstrated reduced O_2 yields in resistant chloroplasts of *S. vulgaris*, and abnormal patterns in response to flashing light [8]. Holt et al.

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Abbreviation: PS, photosystem.

[8] suggested that alterations on the reducing (acceptor) side of PS II in the herbicide-binding protein result in lowered O_2 yields of resistant chloroplasts, and that a modification in the O_2-evolving apparatus (donor side of PS II) also might be indicated.

The present study examines in greater detail the O_2-evolving characteristics of chloroplasts of both susceptible and resistant *S. vulgaris* biotypes under varying flash regimes. Our results help clarify the relationship between the modified inhibitor-binding site and photosynthetic O_2 evolution in resistant chloroplasts. Furthermore, a link between reduced chloroplast efficiency in electron transport and reduced quantum yields of CO_2 fixation is suggested.

Materials and Methods

Plant material. The plants used in this study were grown in soil in a temperature-controlled greenhouse as previously described [8]. 5-week-old plants were harvested for chloroplast isolation. Broken, stroma-free chloroplast thylakoid membranes were extracted from both *S. vulgaris* biotypes as described by Stemler [10]. Either fresh or freeze-thawed grana were used. No difference was detected between these preparations.

O_2 evolution in flashing light. A platinum O_2-specific electrode was used to measure O_2 evolution in response to brief light flashes. This apparatus was modeled after that of Joliot and Joliot [11], and has been described in detail by Holt et al. [8]. Aliquots of broken, stroma-free chloroplasts were placed in the reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 100 mM NaCl, and 1 μM gramicidin D as an uncoupler of photophosphorylation. (Gramicidin D was dissolved in methanol and kept in the freezer; total methanol concentration in the reaction mixture was less than 1%.) Chlorophyll concentrations in the samples used averaged 100 μg Chl/ml for each biotype. After the sample had been allowed to equilibrate on the electrode in the dark at 25°C for 10 min, O_2 yields were measured in response to 3-μs xenon light flashes. Experiments were conducted in which the time between flashes was varied and the effect on the yield of O_2 was determined. For experiments in which the time between the first and second flashes was varied, the first flash of a sequence was produced by triggering two lamps simultaneously. This procedure increases the incidence of intensity-dependent double hits on the first flash after darkness. Because of the greater variability encountered in resistant than in susceptible chloroplasts, a larger number of observations was necessary when resistant plastids were used.

Notation

A model describing the kinetics of O_2 evolution has been described by Kok et al. [12] and Joliot and Kok [13]. 'S' represents the general state of the O_2-evolving enzyme, which cycles through five oxidation states, $S_0 \rightarrow S_4$. Four consecutive photoreactions corresponding to the transfer of four electrons cooperate to produce one O_2 molecule. The model may be depicted as follows:

$$S_0 \xrightarrow{h\nu} S'_0 \rightarrow S_1 \xrightarrow{h\nu} S'_1 \rightarrow S_2 \xrightarrow{h\nu} S'_2 \rightarrow S_3 \xrightarrow{h\nu} S'_3 \rightarrow S_4$$

$$\uparrow \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \downarrow$$

$$O_2 + 4H^+ \qquad\qquad\qquad\qquad\qquad\qquad 2H_2O$$

Since experiments were conducted using a xenon flash with a relatively long tail, we assume that double hits, when they occur, are due primarily to S-state recoveries which occur during the flash. There also may be differences between the two biotypes in intrinsic or nonphotochemical double hits, which are independent of flash duration. However, their observation requires special conditions which were not met in our experiments [14]. In order to explain the kinetics of O_2 evolution presented here, we have adopted the series of notations of Bouges-Bocquet [15]. In our experiments, $\Delta t = 1$ s unless otherwise indicated.

Results

$S_2 \rightarrow S_3$ turnover and deactivation

$S'_2 \rightarrow S_3$ turnover was measured by varying the time between the second and third flash (Δt_{23}) after an initial 10-min dark equilibration. Fig. 1 shows the $S'_2 \rightarrow S_3$ transition for *S. vulgaris* chloroplasts as a function of Δt_{23}. The resistant biotype has a faster turnover half-time ($t_{1/2} = 0.80$ ms) than the susceptible biotype ($t_{1/2} = 0.95$ ms).

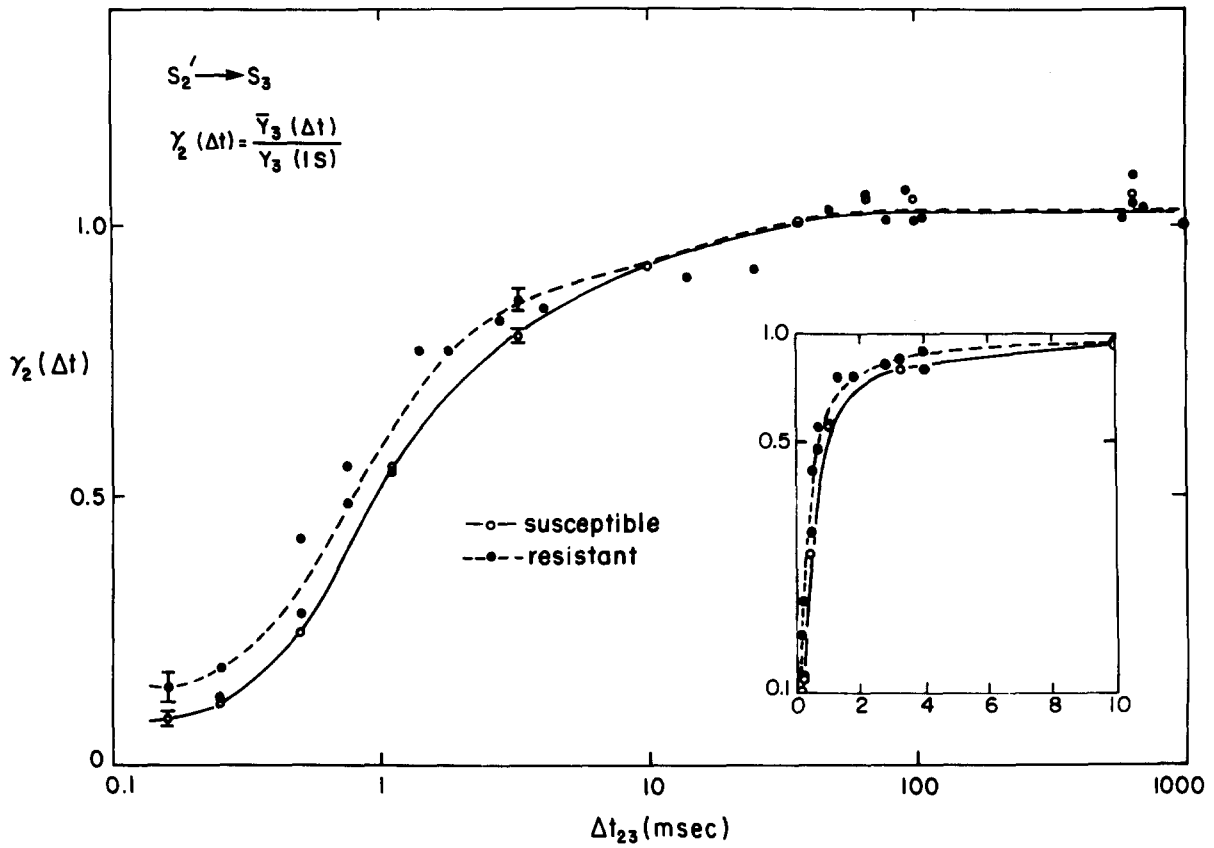
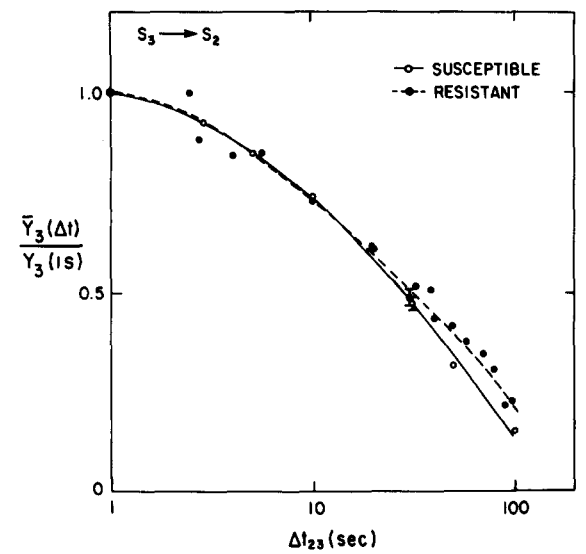


Fig. 1. Recovery to state S_3 by the $S'_2 \rightarrow S_3$ reaction as a function of the time between the second and third flash (Δt_{23}) in *S. vulgaris* chloroplasts. Other flashes are 1 s apart. $\gamma_2(\Delta t)$ is the probability that the $S'_2 \rightarrow S_3$ reaction will occur in the time Δt after a flash. (○) Susceptible, (●) resistant. Error bars represent two standard deviations about the means of five measurements.



In addition, at $\Delta t_{23} = 0.16$ ms, the shortest Δt measured, $\gamma_2(\Delta t)$ is 67% higher in resistant than in susceptible chloroplasts. Rapid turnover of the $S'_2 \rightarrow S_3$ transition in this brief time interval could be responsible for the larger percentage of double hits occurring in resistant chloroplasts which we reported previously [8].

Fig. 2 shows $S_3 \rightarrow S_2$ deactivation after the second flash, calculated by the same formula as for $S'_2 \rightarrow S_3$ turnover. In both resistant and susceptible

Fig. 2. Deactivation of state S_3 as a function of the time between the second and third flash (Δt_{23}) in *S. vulgaris* chloroplasts. Other flashes are 1 s apart. (○) Susceptible, (●) resistant. Error bars represent two standard deviations about the means of five measurements.

chloroplasts, deactivation proceeds at the same rate, with a half-time of approx. 30 s. These results indicate that the kinetics of the $S_2' \rightarrow S_3$ transition are different in susceptible and resistant chloroplasts, when measured after the second flash of a sequence.

$S_i \rightarrow S_2$ turnover and deactivation

The $S_1' \rightarrow S_2$ transition, measured after the first flash, is shown as a function of Δt_{12} in Fig. 3. For susceptible chloroplasts, the half-time for $S_1' \rightarrow S_2$ turnover is 0.60 ms, while the half-time for this transition in resistant chloroplasts is slower, occurring at 1.30 ms. In addition, an important difference between the two curves is seen in the time period 100 ms–1 s. In resistant chloroplasts, an overshoot of the curve occurs as shown on Fig. 3, where $\gamma_1(\Delta t) > 1$. However, the probability of any transition, $S_n' \rightarrow S_{n+1}$, cannot be greater than 1. Therefore, the overshoot observed in Fig. 3 indicates that some $S_2 \rightarrow S_1$ deactivation must have occurred before 1 s, such that the Y_3(1 s) yield is not maximal. From the formula used to calculate $\gamma_1(\Delta t)$ (shown in Fig. 3), it can be seen that if the denominator Y_3(1 s) is too low, then $\gamma_1(\Delta t)$ will be erroneously high, and even apparently exceed 1. If the $\overline{Y}_3(\Delta t)$ value measured at, for example, 500 ms, where $\gamma_1(\Delta t)$ is largest, were used to recalculate $\gamma_1(\Delta t)$, the corrected curve for the $S_1' \rightarrow S_2$ transition of the resistant chloroplasts would be 10–15% lower than the plotted one. 100% turnover would occur around 500 ms and $S_2 \rightarrow S_1$ deactivation would begin before 1 s. Furthermore, it is possible that between 100 ms and 1 s, the processes of turnover and deactivation are competing in the resistant chloroplasts, so that a true maximum value for Y_3 cannot be obtained. Therefore, calculation of $\gamma_1(\Delta t)$ based on even the maximum ob-

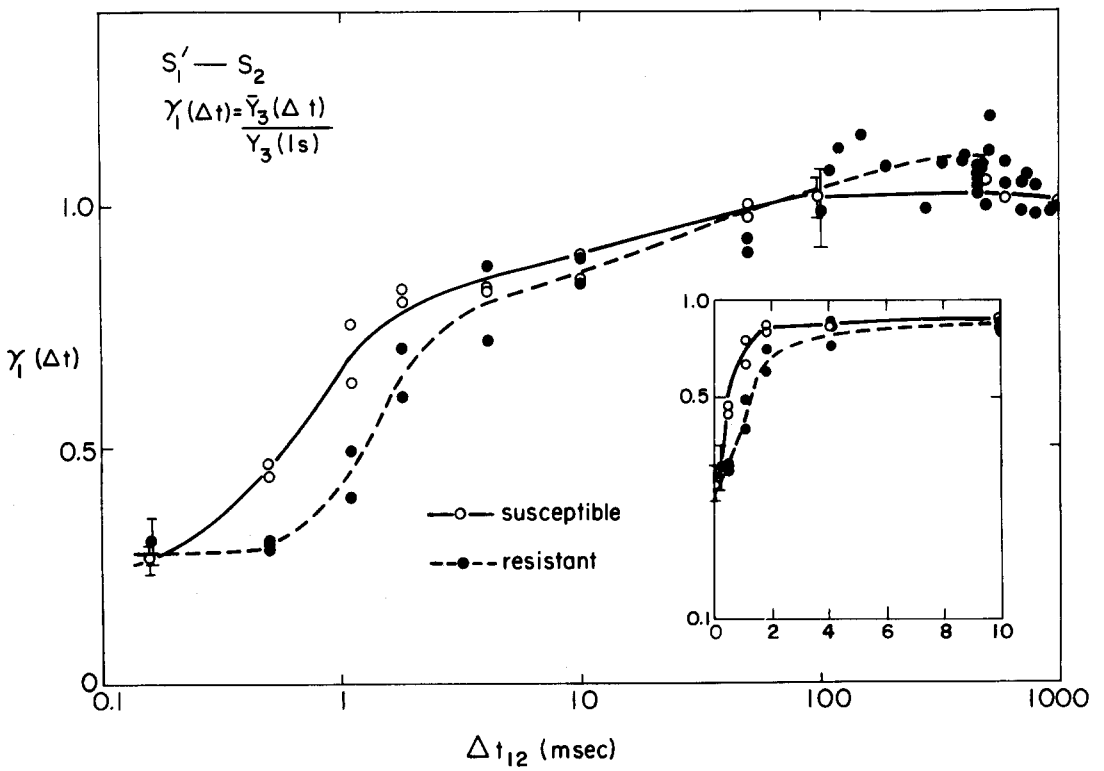


Fig. 3. Recovery to state S_2 by the $S_1' \rightarrow S_2$ reaction as a function of the time between the first and second flash (Δt_{12}) in *S. vulgaris* chloroplasts. Other flashes are 1 s apart. $\gamma_1(\Delta t)$ is the probability that the $S_1' \rightarrow S_2$ reaction will occur in the time Δt after a flash. (○) Susceptible, (●) resistant. Error bars represent two standard deviations about the means of seven (resistant) or four (susceptible) measurements.

served Y_3 value would result in an overestimation of transition rates in resistant chloroplasts.

This result indicates that the $S'_1 \rightarrow S_2$ transition in resistant chloroplasts is slower following the first flash after dark equilibration than in susceptible chloroplasts. It also appears that S_2 deactivation is more rapid. Fig. 4 shows the deactivation kinetics of $S_2 \rightarrow S_1$ after the first flash for chloroplasts of both biotypes, calculated in the same manner as $S'_1 \rightarrow S_2$ turnover. It is clear that S_2 deactivation proceeds significantly faster in resistant chloroplasts ($t_{1/2} = 15$ s) than in susceptible ones ($t_{1/2} = 40$ s). However, just as in the calculations for $S'_1 \rightarrow S_2$ turnover kinetics, the value for $Y_3(1\text{ s})$ for the resistant chloroplasts is not maximal. Therefore, S_2 deactivation in resistant chloroplasts is even faster than Fig. 4 indicates.

An interesting implication of this result concerns the higher incidence of double hits we reported for resistant than susceptible *S. vulgaris* chloroplasts in Ref. 8. In normal systems, double hits which produce O_2 on flash 2 may occur by two means. A double hit can occur on flash 1 ($S_1 \rightarrow S_2 \rightarrow S_3$) followed by a normal conversion on flash 2 ($S_3 \rightarrow S_4 + O_2$), or a normal conversion

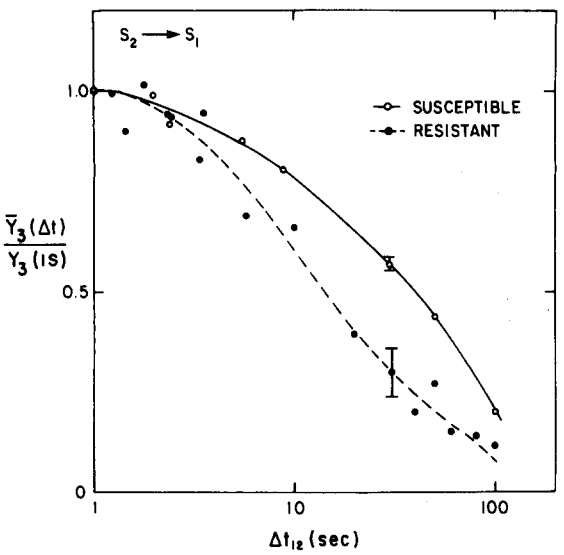


Fig. 4. Deactivation of state S_2 as a function of the time between the first and second flash (Δt_{12}) in *S. vulgaris* chloroplasts. Other flashes are 1 s apart. (○) Susceptible, (●) resistant. Error bars represent two standard deviations about the means of five measurements.

can occur on flash 1 ($S_1 \rightarrow S_2$) followed by a double hit on flash 2 ($S_2 \rightarrow S_3 \rightarrow S_4 + O_2$) [16]. However, in resistant chloroplasts, since $S'_1 \rightarrow S_2$ turnover occurs slowly, double hits will occur with less frequency on flash 1. Furthermore, $S'_2 \rightarrow S_3$ turnover is more rapid than in susceptible chloroplasts, so that double hits are more likely to occur on the second flash after a dark period. Therefore, at least in resistant plants, the component of double hits which is due to rapid S_2 turnover makes up a larger proportion of the O_2 yield on flash 2, compared to the component resulting from S_1 turnover.

$S'_0 \rightarrow S_1$ turnover

The rate of the $S'_0 \rightarrow S_1$ transition is determined from experiments in which the time between the first and second flash is varied. $\gamma_0(\Delta t)$ encompasses the approx. 25% of the reaction centers in state S_0 after a 10 min dark period which yield O_2 on flash 4. These results are shown in Fig. 5. The half-time for the $S'_0 \rightarrow S_1$ transition in susceptible chloroplasts is 0.50 ms, and turnover is complete by 100 ms. Resistant chloroplasts show a slower $t_{1/2}$ for $S'_0 \rightarrow S_1$ turnover (1.40 ms). In addition, for resistant chloroplasts from 100 ms to 1 s the overshoot of the $\gamma_0(\Delta t) = 1$ line is observed, just as in the $S'_1 \rightarrow S_2$ transition kinetics on the first flash (Fig. 3).

It is currently believed that while S_0 and S_1 are in equilibrium in the dark, no net deactivation of S_1 to S_0 takes place [15,16]. Therefore, the overshoot of the curve for $S'_0 \rightarrow S_1$ turnover in resistant chloroplasts is not due to early S_1 deactivation before 1 s. This apparent greater than 100% turnover may be attributed to the rapid deactivation of S_2 to S_1 after the first flash in resistant chloroplasts, demonstrated in Figs. 3 and 4. Rapid S_2 deactivation adds to the S_1 population present at the second flash, and shows up on flash 4 as apparent rapid turnover of $S'_0 \rightarrow S_1$ after flash 1 [$\gamma_0(\Delta t)$]. Analysis of transition kinetics and the formula for calculation of γ_0 indicate that since rapid S_2 deactivation 1 s after flash 1 results in a less than maximal $Y_3(1\text{ s})$ and a slightly enriched $Y_4(1\text{ s})$, the net effect on the calculation for $\gamma_0(\Delta t)$ is an artificial increase. The curve for resistant chloroplasts in Fig. 5, like Fig. 3, should be corrected downward when the proper $Y_3(\Delta t)$ and

$Y_4(\Delta t)$ values are applied. Therefore, $S'_0 \rightarrow S_1$ turnover after flash 1 proceeds more slowly in resistant than in susceptible chloroplasts.

The results of Figs. 1–5 indicate that on the first flash after 10 min of dark, the transitions $S'_0 \rightarrow S_1$ and $S'_1 \rightarrow S_2$ proceed slowly in resistant chloroplasts. Those states in S_1 that turn over to S_2 deactivate quickly to S_1 again, relative to these same photoconversions in susceptible chloroplasts. Furthermore, after a second flash, the $S'_2 \rightarrow S_3$ transition is more rapid in resistant than in susceptible chloroplasts, while $S_3 \rightarrow S_2$ deactivation proceeds at the same rate in both.

Steady-state measurements

To determine if the same S-state kinetics of O_2 evolution are observable at steady state as after a period of dark, measurements were made varying the time between flashes after steady state was reached. Steady-state $S'_2 \rightarrow S_3$ turnover for both biotypes is shown in Fig. 6. At steady state, any flash reflects the S_3 population immediately before the flash. O_2 yield after the double flash, $Y_{ss+1}(\Delta t)$, occurs as a single spike on the chart paper at $\Delta t < 10$ ms. This yield equals the sum of $S'_3 \rightarrow S_0$ on the first flash of the doublet, plus $S'_2 \rightarrow S_3$ reactions completed in Δt. Therefore, to represent only the $S'_2 \rightarrow S_3$ transition, the formula (shown in Fig. 6) is corrected where appropriate by subtracting Y_{ss}.

Resistant chloroplasts have a half-time for $S'_2 \rightarrow S_3$ turnover at steady-state nearly identical to that after the second flash ($t_{1/2} = 0.70$ ms at steady state, $t_{1/2} = 0.80$ ms after second flash). In both instances, 100% turnover has occurred by 30 ms. Susceptible chloroplasts, however, show very different $S'_2 \rightarrow S_3$ transition kinetics at steady state than after the second flash of a sequence. Here,

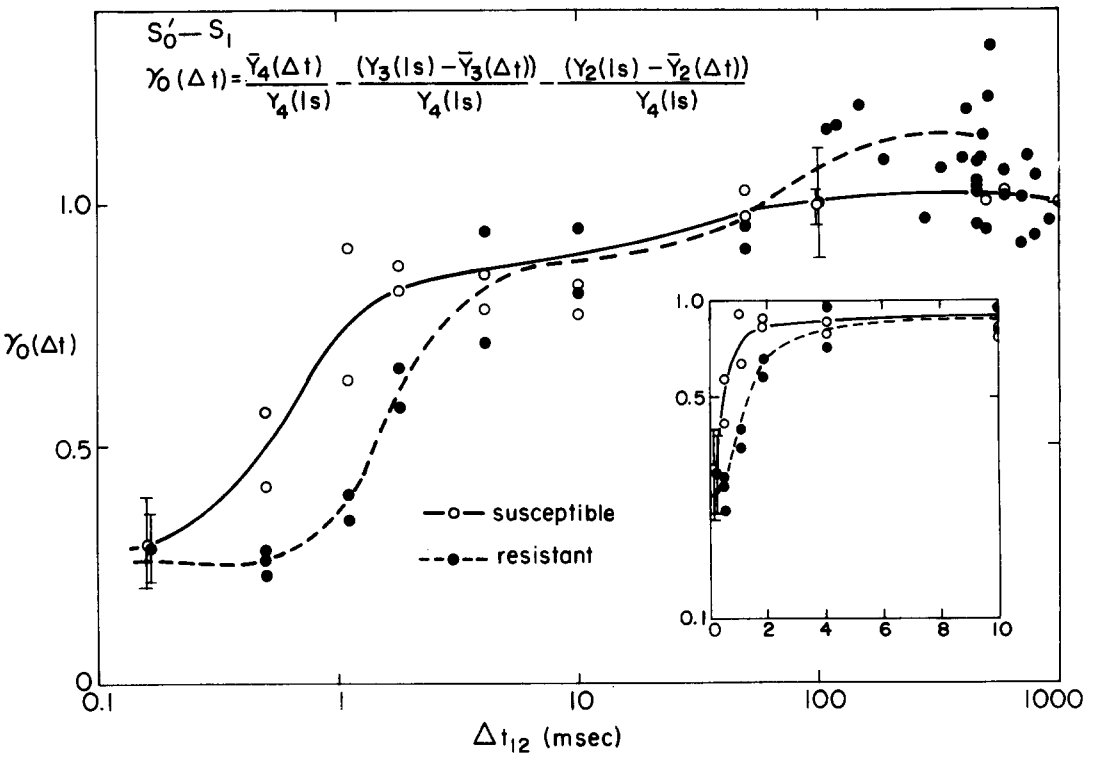


Fig. 5. Recovery to state S_1 by the $S'_0 \rightarrow S_1$ reaction as a function of the time between the first and second flash (Δt_{12}) in *S. vulgaris* chloroplasts. Other flashes are 1 s apart. $\gamma_0(\Delta t)$ is the probability that the $S'_0 \rightarrow S_1$ reaction will occur in the time Δt after a flash. (○) Susceptible, (●) resistant. Error bars represent two standard deviations about the means of seven (resistant) or four (susceptible) measurements.

$t_{1/2} = 2.5$ ms, compared to $t_{1/2}$ after the second flash of 0.95 ms, and full $S_2' \rightarrow S_3$ turnover at steady state has not occurred until approx. 100 ms after Δt.

This commonly observed phenomenon of slow S-state turnover at steady state in normal chloroplasts is generally attributed to a reducing side effect. As the plastoquinone pool fills with electrons after many light flashes, recovery of the PS II reaction centers may become slower following a flash due to reduction of the plastoquinone pool [17]. Discrepancies between our steady-state data for susceptible chloroplasts and previous findings of a biphasic exponential rise with a large fast component in normal chloroplasts are probably due to the state of the plastoquinone pool [18]. Those authors added ferricyanide to the chloroplast suspension, maintaining the plastoquinone pool in an oxidized state, which we did not do. The resistant chloroplasts do not demonstrate the limiting effect of a reduced plastoquinone pool as do the susceptible ones. The reason for this difference is not clear. Most likely, fewer electrons per flash enter the plastoquinone pool in the resistant chloroplasts so that the reduction level of plastoquinone remains low. Other explanations are possible, however. The resistant chloroplasts could have a larger plastoquinone pool, or the rate of removal of electrons from plastoquinone could be different from that of the susceptible ones.

$S_3 + S_2$ deactivation also may be calculated from Δt experiments at steady state by the formula $Y_{ss+1}(\Delta t)/Y_{ss}$. Fig. 7 shows S_3 deactivation curves for both biotypes to be similar, with a $t_{1/2}$ of 20 s. This is slightly faster than S_3 deactivation after the second flash (see Fig. 2), and may be attributed to higher reduction levels of intersystem intermediates. Unfortunately, attempts to calculate other S-state parameters at steady state give meaningless results because of the high probability

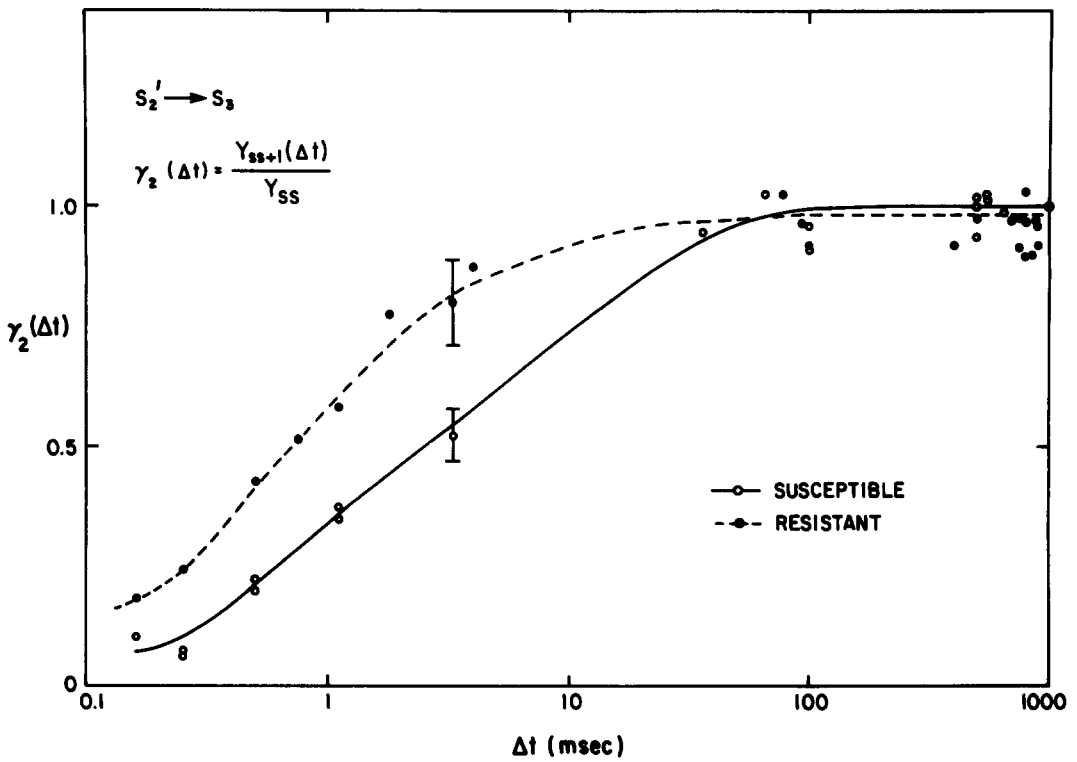


Fig. 6. Recovery to state S_3 from steady state as a function of the time (Δt) between two flashes at steady-state (approximately 22nd and 23rd) in *S. vulgaris* chloroplasts. Other flashes are 1 sec apart. $\gamma_2(\Delta t)$ is the probability that the $S_2' \rightarrow S_3$ reaction will occur in the time Δt after a flash. (○) susceptible, (●) resistant. Error bars represent two standard deviations about the means of 10 (resistant) or 5 (susceptible) measurements.

of nonrandom S-state distributions at steady state in resistant chloroplasts. This is most likely due to rapid decay of the S_2 state, which could result in a consistently higher proportion of S_1 at steady state.

Δt = 1 s measurement

Our results indicate that in resistant chloroplasts, the $S_1 \rightarrow S_2$ transition is abnormal in turnover and deactivation rates at least on the first flash. Additionally, the $S'_0 \rightarrow S_1$ transition is abnormal on flash 1, while $S_2 \rightarrow S_3$ turnover and deactivation proceed normally on flash 2. These findings suggest that if abnormal $S_1 \rightarrow S_2$ events continue to occur after each flash, Δt measurements made at 1-s intervals should not yield maximal O_2 amounts due to some $S_2 \rightarrow S_1$ deactivation before 1 s. Therefore, experiments were conducted in which sequences of 15 equally spaced flashes were given to chloroplasts of both biotypes. Values for Δt used were 500 ms, 1 s and 2.1 s. In this way, any rapid S-state deactivation in resistant chloroplasts by 1 s after each flash should be detected by larger O_2 yields at 500 ms and smaller O_2 yields at 2.1 s, relative to yields at 1-s intervals. These results are shown in Fig. 8. For susceptible chloroplasts (Fig. 8a), as expected from S-state turnover and deactivation kinetics, no significant difference is found between Δt measurements of 500 ms, 1 s and 2.1 s. In these chloroplasts a broad plateau exists in S-state turnover and deactivation curves from 500 ms to 2.1 s, where turnover is complete and deactivation is minimal. Therefore, Δt = 1 s is an appropriate time interval at which to present normal PS II reaction centers with light flashes to achieve maximal O_2 yields.

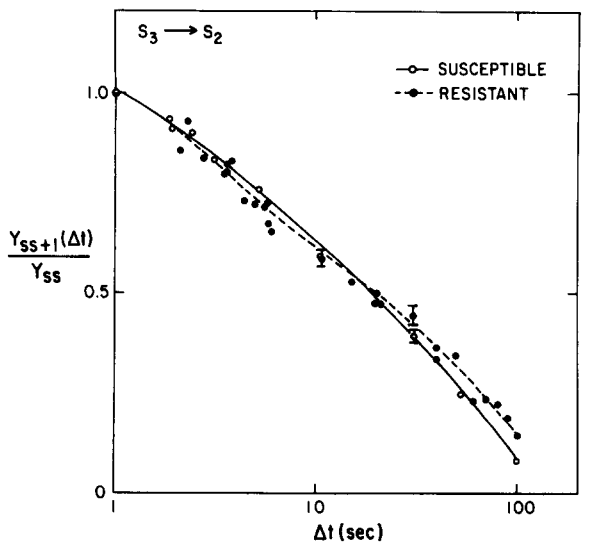


Fig. 7. Deactivation of state S_3 from steady state as a function of the time (Δt) between two flashes at steady state (approx. 22nd and 23rd) in *S. vulgaris* chloroplasts. Other flashes are 1 s apart. (○) Susceptible, (●) resistant. Error bars represent two standard deviations about the means of five measurements.

Resistant chloroplasts are quite different, however. $Y_3(\Delta t)$ values at 1-s intervals are significantly lower than at 500-ms intervals, and at 2.1-s intervals are lowest of all (Fig. 8b). These results indicate that 500 ms after a flash, the ratio of percent recovery/percent deactivation is maximal. Significant deactivation has occurred at 1 s, and even more at 2.1-s intervals after the second flash. Since $S_2 \rightarrow S_3$ turnover and deactivation kinetics were normal in resistant chloroplasts, it seems that rapid S_2 deactivation must account for lower flash yields of oxygen in the resistant chloroplasts.

In addition, in resistant chloroplasts all O_2 yields beyond $Y_3(\Delta t)$ at 500-ms intervals are slightly higher than at 1 s, and yields at 500 ms and at 1 s are significantly higher than at 2.1-s intervals between flashes. It is probable that rapid S_2 deactivation occurs after 500 ms following every flash, resulting in reduced O_2 yields per flash in resistant chloroplasts when $\Delta t > 500$ ms. However, the $S_1 \rightarrow S_2$ transition appears to be more altered in turnover and deactivation after the first flash, since the greatest difference between yields at 500-ms and 1-s intervals occurs in $Y_3(\Delta t)$. Certainly, after all flashes, rapid S_2 deactivation has begun by 2.1 s, a phenomenon not seen in susceptible chloroplasts. These results suggest that a back-flow of electrons to S_2 causing deactivation to S_1 occurs with greater frequency in resistant than in susceptible chloroplasts.

Discussion

We have demonstrated that in resistant chloroplasts of *S. vulgaris*, the first flash of a sequence after dark adaptation is followed by slower rates of the $S'_0 \rightarrow S_1$ and $S'_1 \rightarrow S_2$ transitions by O_2-evolving PS II complexes. In contrast, the rate of the $S'_2 \rightarrow S_3$ transition following the second flash is actually faster in resistant chloroplasts. From mea-

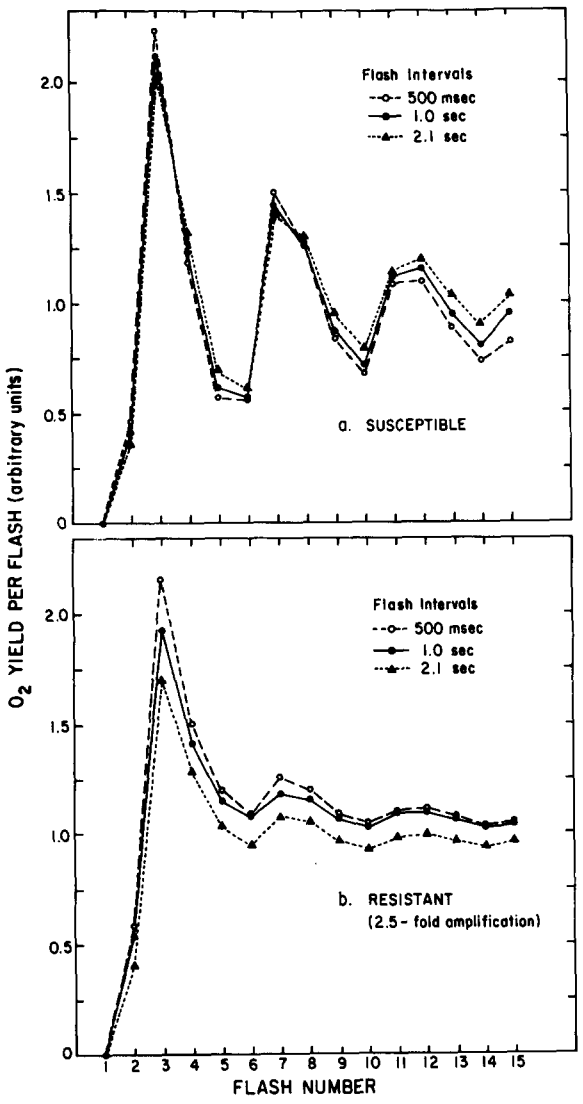


Fig. 8. Effect of the interval between flashes on the oxygen yield per flash in *S. vulgaris* chloroplasts. Saturating 3-μs flashes were given after 10 min dark at 25°C at the intervals 500 ms (O— — — O), 1 s (●———●) and 2 s (▲· · · · · ▲). (a) Susceptible chloroplasts. Each point is an average of seven measurements. Differences in O_2 yield between time intervals are not significant at $P = 0.05$. (b) Resistant chloroplasts. Each point is an average of 10 measurements. Yields for resistant chloroplasts are amplified 2.5-times. Differences in O_2 yield between the 2.1-s and 1-s, and between the 2.1-s and 500-ms intervals are significant at $P = 0.05$ for flashes 2–11. Differences in O_2 yield between the 1-s and 500-ms intervals are significant at $P = 0.05$ for flashes 3–5.

surements of fluorescence decay following a single flash, Kok et al. [12] proposed that dark $S'_n \rightarrow S_{n+1}$ turnovers are limited by the reoxidation rate of Q^-, the primary electron acceptor of PS II. Fluorescence studies by Pfister and Arntzen [7] with triazine-resistant and -susceptible weed biotypes indicated that in resistant chloroplasts, the concentration of reduced Q, Q^-, is higher after dark adaptation, and that the rate constant for Q^- reoxidation is altered in these chloroplasts. These workers suggested a change in the redox potentials, and consequently the reaction kinetics, of the Q^-/B (B^-) pair in resistant chloroplasts [7]. Carrying this explanation further, if some Q remains in the reduced state in resistant chloroplasts at all times, each light flash will photoconvert a smaller fraction of the light traps. This phenomenon could be one cause for increased misses which we reported earlier [8] in resistant chloroplasts. Another cause of misses could be a rapid deactivation of the S_2 state as reported here. These two sources of misses may well be related, however.

Further evidence linking S-state kinetics on flash 1 with reducing side phenomena comes from the work of Bowes et al. [9]. Reoxidation of Q^- after a single flash was shown to be 10-fold slower in resistant than susceptible chloroplasts of *Amaranthus retroflexus*, which has a mechanism of resistance similar to that of *S. vulgaris* [7]. Furthermore, binary oscillations in the rate of Q^- decay corresponding to the reactions $Q^-B \rightarrow QB^-$ and $Q^-B^- \rightarrow QB^{2-}$ were shown to be of opposite phase in the two biotypes [9]. These results were interpreted to mean that in susceptible chloroplasts the $Q^-B \rightarrow QB^-$ reaction, which occurs on flash 1 after dark adaptation, is faster than $Q^-B^- \rightarrow QB^{2-}$ which occurs on flash 2. In resistant chloroplasts this relationship is reversed ($Q^-B \rightarrow QB^-$ proceeds more slowly relative to $Q^-B^- \rightarrow QB^{2-}$).

Although the species used, conditions, and time course of the experiments of Bowes et al. are not identical to ours, our results are, at least qualitatively, in agreement with their first and second flash fluorescence data. In resistant *S. vulgaris*, the rate of transitions $S'_0 \rightarrow S_1$ and $S'_1 \rightarrow S_2$ after the first flash are much slower than the rate of transition $S'_2 \rightarrow S_3$ which occurs after the second flash. Compared to the same transitions in susceptible

chloroplasts, $S'_0 \rightarrow S_1$ and $S'_1 \rightarrow S_2$ in resistant chloroplasts are much slower, again in agreement with fluorescence decay measurements [9].

The data presented in Fig. 8 show, however, that after every flash of a sequence, reaction centers in state S_2 deactivate to S_1 with greater frequency in resistant than susceptible chloroplasts. Thus, the abnormal PS II kinetics seen in resistant chloroplasts are not simply a first-flash phenomenon, but are characteristics of the system at steady state as well. Unfortunately, it is not fully understood how S_2 (or S_3) deactivation occurs even in normal systems. A common explanation is electron donation from exogenous or endogenous donors. One endogenous electron source could be a large amount of reduced Q^-, which causes back electron flow to the S-states via chlorophyll P-680. The possibility of electron flow back to intermediates on the oxidizing side of PS II has been considered [13,19–21], and the role of back-reactions in delayed light emission has been reviewed in detail elsewhere [22,23]. A proposed altered redox relationship of Q and B in resistant chloroplasts will give rise to increased back-reactions, since at any time a larger amount of Q^- appears to be present in resistant chloroplasts compared to susceptible ones [9]. There is considerable evidence to suggest that, depending on conditions of measurement, deactivation of state S_2 may be preferentially enhanced many-fold by electron flow from the reducing side of PS II. Work done with inhibitors such as 3-(3,4-dichlorophenyl)-1,1-dimethylurea in *Chlorella* [24] or in chloroplasts [7,17,25], with ADRY reagents (reagents accelerating the deactivation reaction of water-splitting enzyme system Y) [24,26], and studies of PS II events occurring under anaerobic conditions [17] support this idea.

We therefore propose that in resistant chloroplasts, the following reaction sequence occurs in some PS II complexes after each flash of a series, perhaps reflecting an alteration of the redox potential of Q relative to B (B^-):

$$S_1 \cdots QB \xrightarrow{h\nu} S'_1 \cdots Q^- B \rightarrow S_2 \cdots QB^-$$

$$\rightarrow S_2 \cdots Q^- B \rightarrow S_1 \cdots QB$$

This chain of events lowers O_2 yields per flash by reversing a certain portion of forward electron movements, and results in decreased quantum efficiency of the photosynthetic light reactions. Evidence that the quantum yield of CO_2 fixation is reduced in resistant chloroplasts [8] supports this conclusion. Resistant *S. vulgaris* chloroplasts appear to be modified from susceptible ones not only in having a high concentration of Q^- in the dark resulting in high initial fluorescence, but also in increased incidence of back-reactions from the Q^-/B pair to state S_2 of the oxygen-evolving system.

It thus appears that much of the data presented here can be explained by assuming that electron transfer $Q^- \rightarrow B$ (B^-) proceeds more slowly in triazine-resistant chloroplasts, while the back-reaction is favored. However, some of the data presented are difficult to explain by this simple model. It is not clear, for example, why the $S'_1 \rightarrow S_2$ transition appears bi- (or poly-) phasic in normal chloroplasts and markedly so in resistant ones (Fig. 3). It is also puzzling why the $S'_2 \rightarrow S_3$ transition is actually faster in the resistant chloroplasts, particularly in the time period less than 1 ms (Figs. 1 and 6). Recovery rates for both the $S'_1 \rightarrow S_2$ and $S'_2 \rightarrow S_3$ transitions in susceptible chloroplasts were somewhat less than those reported previously for normal chloroplasts [15]. We note that our measurements were made at lower pH and under somewhat different conditions. In light of these complications, it may yet be necessary to propose a more direct modification of the oxygen-evolving mechanism in the triazine-resistant chloroplasts. Until more detailed work is done, we cannot rule out the possibility that such a modification is present.

Our studies with susceptible and resistant biotypes of *S. vulgaris* have focused on elucidating the physiological mechanisms underlying fitness differences between biotypes. We have demonstrated whole plant differences [8], and linked them to intrinsic differences at the level of the chloroplast thylakoid membrane. From this work, a more comprehensive picture has emerged of the relationship of chloroplast function to whole plant performance. Furthermore, resistant plants have properties of the photosynthetic light reactions not demonstrated before in chloroplasts untreated with chemicals or other manipulating agents, and as

such are a valuable tool in the study of photosynthesis itself.

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References

1 Radosevich, S.R. and Appleby, A.P. (1973) Agron, J. 65, 553–555
2 Jensen, K.I.N., Bandeen, J.D. and Souza-Machado, V. (1977) Can. J. Plant Sci. 57, 1169–1177
3 Ryan, G.F. (1970) Weed Sci. 18, 614–616
4 Conard, S.G. and Radosevich, S.R. (1979) J. Appl. Ecol. 16, 171–177
5 Arntzen, C.J., Ditto, C. and Brewer, P. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 278–282
6 Radosevich, S.R., Steinback, K.E. and Arntzen, C.J. (1979) Weed Sci. 27, 216–218
7 Pfister, K. and Arntzen, C.J. (1979) Z. Naturforsch. 34C, 996–1009
8 Holt, J. Sims, Stemler, A.J. and Radosevich, S.R. (1981) Plant Physiol. 67, 744–748
9 Bowes, J., Crofts, A.R. and Arntzen, C.J. (1980) Arch. Biochem. Biophys. 200, 303–308
10 Stemler, A. (1980) Plant Physiol. 65, 1160–1165
11 Joliot, P. and Joliot, A. (1968) Biochim. Biophys. Acta 153, 625–634
12 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475
13 Joliot, P. and Kok, B. (1975) Bioenergetics of Photosynthesis (Govindgee, ed.), pp. 387–412, Academic Press, New York
14 Jursinic, P. (1981) Biochim. Biophys. Acta 635, 38–52
15 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 292, 772–785
16 Forbush, B., Kok, B. and McGloin, M. (1971) Photochem. Photobiol. 14, 307–321
17 Diner, B.A. and Joliot, P. (1977) in Photosynthesis I (Trebst, A. and Avron, M., eds.), pp. 187–205, Springer, Berlin
18 Vater, J., Renger, G., Stiehl, H.H. and Witt, H.T. (1968) Naturwissenschaft 55, 220–221
19 Radmer, R. and Kok, B. (1975) Annu. Rev. Biochem. 44, 409–433
20 Velthuys, B.R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85–94
21 Heath, R.L. (1970) Biophys. J. 10, 1173–1188
22 Amesz, J. and Van Gorkom, H.J. (1978) Annu. Rev. Plant Physiol. 29, 47–66
23 Vierke, G. (1980) Z. Naturforsch. 35C, 638–644
24 Lavorel, J. (1980) Biochim. Biophys. Acta 590, 385–399
25 Wollman, F.-A. (1978) Biochim. Biophys. Acta 503, 263–273
26 Renger, G., Bouges-Bocquet, B. and Delosme, R. (1973) Biochim. Biophys. Acta 292, 796–807