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# Effects on Photosystem II Function, Photoinhibition, and Plant Performance of the Spontaneous Mutation of Serine-264 in the Photosystem II Reaction Center D1 Protein in Triazine-Resistant *Brassica napus* L.[1]

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**Wild-type and an atrazine-resistant biotype of *Brassica napus*, in which a glycine is substituted for the serine-264 of the D1 protein, were grown over a wide range of constant irradiances in a growth cabinet. In the absence of serine-264, the function of photosystem II (PSII) was changed as reflected by changes in chlorophyll fluorescence parameters and in photosynthetic oxygen-evolving activity. The photochemical quenching coefficient was lower, showing that a larger proportion of the primary quinone acceptor is reduced at all irradiances. At low actinic irradiances, the nonphotochemical quenching coefficient was higher, showing a greater tendency for heat emission. Decreased rates of light-limited photosynthesis (quantum yield) and lower oxygen yields per single-turnover flash were also observed. These changes were observed even when the plants had been grown under low irradiances, indicating that the changes in PSII function are direct and not consequences of photoinhibition. In spite of the lowered PSII efficiency under light-limiting conditions, the light-saturated photosynthesis rate of the atrazine-resistant mutant was similar to that of the wild type. An enhanced susceptibility to photoinhibition was observed for the atrazine-resistant biotype compared to the wild type when plants were grown under high and intermediate, but not low, irradiance. We conclude that the replacement of serine by glycine in the D1 protein has a direct effect on PSII function, which in turn causes increased photoinhibitory damage and increased rates of turnover of the D1 protein. Both the intrinsic lowering of light-limited photosynthetic efficiency and the increased sensitivity to photoinhibition probably contribute to reduced crop yields in the field, to different extents, depending on growth conditions.**

The PSII reaction center of green plants performs one of the key steps in the process of photosynthetic energy conversion. The heart of the PSII reaction center comprises a heterodimer of D1 and D2 proteins, with known primary sequences, to which all of the redox components are bound, as has been recently deduced from the close analogy with the crystallized reaction center of photosynthetic bacteria (Hansson and Wydrzynski, 1990; Andersson and Styring, 1991). Upon absorption of a photon by P680, charge separation takes place, which can result either in photochemistry if stabilized by $Q_A$ reduction or in heat emission via a back reaction. After transfer of the electron from $Q_A^-$ to $Q_B$ and then to the plastoquinone pool, the reaction center is ready to utilize a second photon. The D1 protein in the PSII reaction center heterodimer has a light-dependent and rapid rate of turnover (Mattoo et al., 1984), which is related to the phenomenon of photoinhibition (Prasil et al., 1992). Continuous repair of PSII is believed to be necessary because of the involvement of highly oxidizing radicals and toxic oxygen species (Barber and Andersson, 1991; Prasil et al., 1992).

The study of effects of single amino acid substitutions has become an important approach for elucidating molecular mechanisms by which a particular protein functions. One specific alteration in the reaction center of PSII that offers the possibility for such studies in higher plants is the spontaneous mutation of the D1 polypeptide, which prevents binding of atrazine and other triazines: this substitution of D1-Ser$^{264}$ by Gly has been detected in several weed species that survive the extensive use of the PSII herbicide atrazine (Hirschberg et al., 1987). This atrazine-resistant trait has been bred into crop plants for potential use in selective weed control, in spite of the associated problem of reduced performance shown by atrazine-resistant plants. The D1-Ser$^{264}$ is involved in both atrazine and plastoquinone binding in the $Q_B$-binding niche of D1 protein (Trebst, 1987); its absence not only prevents binding of atrazine but also reduces binding of plastoquinone molecules. The reduced binding of plastoquinone, in turn, slows down PSII electron transport in atrazine-resistant biotypes by decreasing the rate of electron transfer between $Q_A$ and $Q_B$ as shown by measurements of the decay of Chl fluorescence yield associated with $Q_A^-$ reoxidation (Bowes et al., 1980).

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Abbreviations: D1 and D2, diffusely staining $Q_B$- and $Q_A$-binding proteins of PSII heterodimer, respectively; $F$, steady-state Chl fluorescence during illumination; $F_v$, variable fluorescence; $F_m$, maximum fluorescence; $F'_v$ and $F'_m$, variable and maximum fluorescence during steady illumination, respectively; P680 and P700, primary electron donor of PSII and PSI, respectively; $P_{max}$, maximal rate of photosynthesis; $Q_A$ and $Q_B$, primary and secondary quinone electron acceptors in PSII, respectively; $q_N$, nonphotochemical quenching coefficient; $q_P$, photochemical quenching coefficient.

It was recently discovered (Hart and Stemler, 1990b) that an atrazine-resistant biotype of *Brassica napus* was more sensitive to photoinhibition than the wild type. In a second study, the authors found less reduction in quantum yield between the atrazine-resistant biotype and wild type when grown under low light than when grown under high light (Hart and Stemler, 1990a) and suggested that the reduction in quantum yield and photosynthesis of resistant plants is an indirect effect of photoinhibition.

If the molecular mechanisms of both photoinhibition and of PSII function are to be elucidated, it is important to establish the molecular mechanisms for the effects of this mutation. To gain further insight into these mechanisms, we have grown D1-Ser$^{264}$ mutants of *B. napus* (Reith and Straus, 1987) over a wide range of constant irradiances in a growth cabinet. We have analyzed the direct effects of the change from Ser$^{264}$ to Gly on the function of PSII in leaves, susceptibility to photoinhibition, acclimation, and photosynthetic performance as functions of the growth irradiance. We conclude that the lower crop yield of atrazine-resistant plants is probably caused by two distinct effects of the mutation, which are partly related. First, the mutation causes a direct effect on PSII photochemistry, which can be observed at all growth irradiances and causes an intrinsic lowering of photosynthetic efficiency of PSII, which reduces light-limited, but not light-saturated, photosynthesis. Second, the altered PSII photochemistry also gives rise to an increased susceptibility to photoinhibition, which probably affects the crop yield at higher growth irradiances. We conclude that the increased susceptibility to photoinhibition is a consequence, rather than the cause, of the altered PSII chemistry.

## MATERIALS AND METHODS

### Plant Material

Seeds of atrazine-susceptible (S, wild type) and atrazine-resistant (R) biotypes of *Brassica napus* (cv Triton [Reith and Straus, 1987]) were sown in pots with vermiculite and grown at low, intermediate, and high light intensities (50 and 150, 350, and 600–750 $\mu$mol photons $m^{-2}$ $s^{-1}$ [i.e. $\mu$E $m^{-2}$ $s^{-1}$]) in a growth cabinet with a photoperiod of 14 h. The temperature was 23°C during the day and 17°C at night. Plants were given Hoagland solution in the morning and water in the afternoon. Leaf function (leaf numbers 3–4) was determined between d 21 and 28 after sowing. For evaluation of the photosynthetic performance of atrazine-resistant biotypes versus the wild type at different growth irradiances, plants were harvested on d 28, and the fresh weight of the shoots was determined and expressed in grams per plant.

### Determination of Photosynthetic Components and Activities

Chl fluorescence yield measurements were performed on individual leaves (in air) attached to the plant with a PAM fluorometer (Heinz Walz, Effeltrich, Germany). Each leaf was sandwiched between the end of a multifurcated light guide on the upper side and a piece of sponge on the lower side. Calculations of $q_P$ and $q_N$ were made, and the symbols were adopted as described by van Kooten and Snel (1990). The quantum efficiency of photochemistry, averaged over all PSII reaction centers near the measured surface, was calculated as $1 - F/F'_m$ according to the method of Genty et al. (1989).

To estimate the reoxidation kinetics of $Q_A^-$, we measured the overall half-time for the multiphasic decay of Chl fluorescence yield after a xenon flash (XST 103 xenon flash lamp, supplied with the PAM fluorometer). Leaves were incubated in the dark for 20 min. With the weak modulated light turned on (1.6 kHz), a level of fluorescence yield corresponding to $F_o$ was displayed on a digital storage oscilloscope (type 1421; Gould). When a "start" trigger was applied to the xenon lamp, the modulated frequency was automatically switched to 100 kHz to improve the signal-to-noise ratio. The increase in Chl fluorescence yield induced by the flash, together with the subsequent decline after the flash, was recorded by the storage oscilloscope and subsequently displayed on a chart recorder.

For rapid measurements of $F_v/F_m$, a plant efficiency analyzer (Hansatech, King's Lynn, UK) was used. The leaf discs were incubated in the dark for 30 min in the leaf clips before a 5-s light pulse (1500 $\mu$E $m^{-2}$ $s^{-1}$) was supplied by an array of red light-emitting diodes. The rapid turn-on of the light-emitting diodes allowed the accurate determination of $F_o$ and, hence, $F_v$.

The light-limited quantum yields and the $CO_2$- and light-saturated rates of oxygen evolution were determined with a leaf disc oxygen electrode in 1% $CO_2$ according to the method of Ball et al. (1987). The oxygen yields per single-turnover flash were also determined with a leaf disc oxygen electrode after subtracting a small heating artifact signal, according to the procedure of Chow et al. (1991). These oxygen yields give the numbers of PSII reaction centers effective in stable charge stabilization. Chl content and Chl *a*/Chl *b* ratios were determined as described by Ball et al. (1987).

Chloroplasts were isolated as previously described (Chow and Hope, 1987). Determinations of DCMU-binding sites, Cyt *f*, and P700 contents per mol of Chl in isolated thylakoids were made as described by Chow and Hope (1987).

### Photoinhibitory Treatment and Recovery

Leaf discs (12 mm diameter) with their upper surfaces exposed to air were floated on water in a Petri dish placed in a large thermostat-controlled water bath at 23°C (Oquist et al., 1992). The leaf discs were exposed for up to 4 h to 1600 $\mu$E $m^{-2}$ $s^{-1}$ supplied by a 575-W Brite Arc HMI studio lamp (Sylvania, GTE Products Corp.) that was fitted with a heat-reflecting filter (Schott; heat-reflecting glass, type 115). After different periods of photoinhibition, leaf discs were dark adapted for 30 min before assay for PSII activity by measuring the Chl fluorescence ratio, $F_v/F_m$, as described above. After photoinhibition, leaf discs were allowed to recover at 100 $\mu$E $m^{-2}$ $s^{-1}$.

## RESULTS

### Effects of the Mutation on PSII Function in Leaves of the Atrazine-Resistant Biotype Leaves

Analysis of Chl fluorescence decay following flash illumination of atrazine-resistant biotypes of several different spe-

cies (Pfister and Arntzen, 1979; Bowes et al., 1980; Jursinic and Pearcy, 1988; Hart and Stemler 1990a; Jansen and Pfister, 1990) has indicated that electron transfer from $Q_A$ to $Q_B$ occurs more slowly than in the respective wild types. We measured the overall half-time of the multiphasic fluorescence decay on our plant material after giving a single xenon flash to intact *B. napus* leaves that had been dark treated for 20 min. The overall half-time was 0.37 ± 0.01 ms for the wild type and 3.2 ± 0.2 ms for the atrazine-resistant biotype when grown under 700 $\mu$E m$^{-2}$ s$^{-1}$. When plants were grown under 50 $\mu$E m$^{-2}$ s$^{-1}$, the overall half-time was 0.35 ± 0.01 ms for the wild-type and 2.5 ± 0.3 ms for the atrazine-resistant biotype.

When modulated excitation light is used, fluorescence characteristics can be followed during illumination with background actinic light at different irradiances. A measure of the oxidation state of $Q_A$ is given by the Chl fluorescence photochemical quenching parameter, $q_P$ (Genty et al., 1989; van Kooten and Snel, 1990). Figure 1 shows a comparison between atrazine-resistant biotypes and the wild types of *B. napus* with respect to $q_P$ measured in different background irradiances. It shows that the fraction of open PSII centers, as indicated by $q_P$, decreased with increasing actinic light in leaves of plants grown under 50 $\mu$E m$^{-2}$ s$^{-1}$; furthermore, the atrazine-resistant biotype differs from the wild type in that $q_P$ is approximately 10 to 15% lower at all incident actinic irradiances. Figure 1B shows that, when the *B. napus* plants

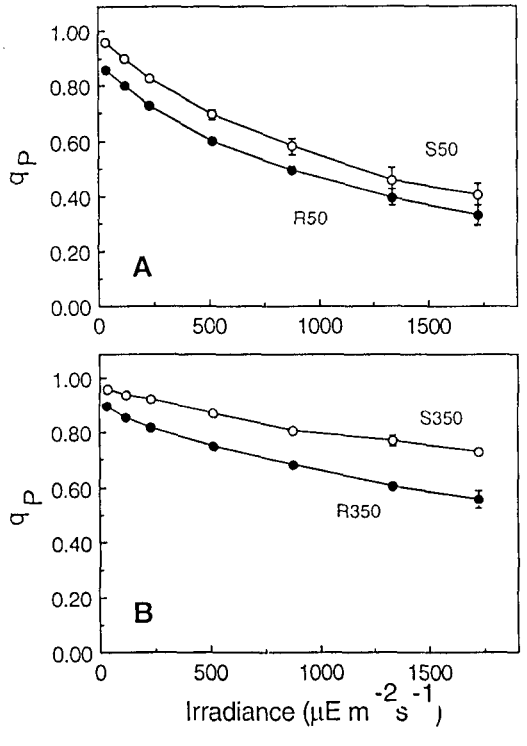


**Figure 1.** The oxidation state of $Q_A$ in PSII, assayed by $q_P$ as a function of incident irradiance to which intact leaves of wild type (S) and the atrazine-resistant biotype (R) of *B. napus* were exposed. The plants were grown under 50 (A) or 350 (B) $\mu$E m$^{-2}$ s$^{-1}$. The values are means ± SE for four leaves.

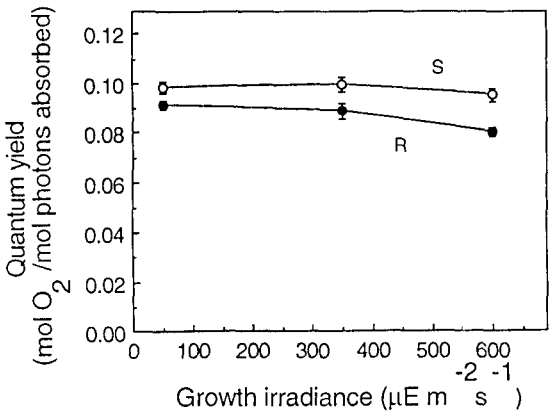


**Figure 2.** The quantum yield of oxygen evolution based on absorbed photons and measured in air containing 1% $CO_2$ and limited light. Wild type (S) and the atrazine-resistant biotype (R) of *B. napus* were grown under the irradiances indicated. The values are means ± SE for four or five leaves.

were grown under 350 $\mu$E m$^{-2}$ s$^{-1}$, the atrazine-resistant biotype again differs from the wild type in terms of $q_P$ at all incident irradiances. It should be noted that plants that are grown under a higher irradiance (Fig. 1B) show a better ability to maintain a high $q_P$ and avoid overreduction of the acceptor side compared to plants grown under lower irradiance (Fig. 1A).

The significantly lower $q_P$ in our atrazine-resistant biotype than in atrazine-susceptible biotype, even at low actinic irradiances, implies that a fraction of the PSII traps are closed during steady-state illumination. These closed traps, unable to undergo stable charge separation and to take part in linear photosynthetic electron transport, should give rise to a loss of light-limited quantum efficiency of photochemistry, even for plants grown at a growth irradiance as low as 50 $\mu$E m$^{-2}$ s$^{-1}$ (Fig. 1A). However, this seems contradictory to the conclusions by Hart and Stemler, who found a lower quantum yield only in high-light-grown triazine-resistant *B. napus* plants (Hart and Stemler, 1990a) and suggested that the lower quantum yield for atrazine-resistant plants is a consequence of their greater susceptibility to photoinhibition (Hart and Stemler, 1990b). Other investigators also reported lower quantum yields of oxygen evolution or carbon assimilation in triazine-resistant biotypes of *Amaranthus hybridus* (Ort et al., 1983), *Senecio vulgaris* (Holt et al., 1981; Ireland et al., 1988), and *B. napus* (Jursinic and Pearcy, 1988), but in none of these studies were the plants grown under low light.

Therefore, in addition to the $q_P$ measurements in Figure 1, we measured directly the quantum yield, both for plants grown at high (600), intermediate (350), and very low (50 $\mu$E m$^{-2}$ s$^{-1}$) irradiance. Figure 2 shows that the light-limited quantum yields of the wild type were close to 0.10 mol of oxygen mol$^{-1}$ of photons absorbed at all growth irradiances and that for the atrazine-resistant biotypes, the quantum yield is lower than for the wild type at all growth irradiances. However, the difference between the atrazine-resistant and -susceptible biotypes increased somewhat with the growth irradiance: at 50 $\mu$E m$^{-2}$ s$^{-1}$, the atrazine-resistant biotype

had a value of 0.091 (92% of the wild type), compared to 0.080 (84% of the wild type) at 600 $\mu$E $m^{-2}$ $s^{-1}$. Thus, in accordance with Hart and Stemler (1990b), the quantum yield is largely decreased in high-light-grown atrazine-resistant plants. However, in contrast to their data, our data in both Figures 1A and 2 show that a decreased quantum yield can also be observed in the material grown under very low light.

To substantiate further the loss of quantum efficiency in atrazine-resistant biotype leaves, we measured the quantum yield of photochemistry during illumination (averaged over all PSII reaction centers near the measured surface) in terms of the Chl fluorescence parameter, $1 - F/F'_m$, of Genty et al. (1989), which is equal to the product of $q_P$ and the fluorescence ratio $F'_v/F'_m$ under different incident irradiances. Relative to the wild type, atrazine-resistant biotypes had lower average quantum yields of PSII photochemistry at all actinic irradiances, even at low irradiance (Fig. 3). Furthermore, this finding also applied to biotypes grown at low growth irradiance. At higher actinic irradiances, the parameter $1 - F/F'_m$ declined because of enhanced energized quenching of excitation energy and a decrease in $q_P$.

We also compared the oxygen yield during repetitive flash illumination for the atrazine-resistant biotype and wild type from different growth irradiances. As shown in Figure 4A, the amount of oxygen produced per flash for the wild type corresponded to about 2.7 mmol of functional PSII per mol of Chl, but for atrazine-resistant biotypes, the values were approximately 25% lower; this is also true for low-light-grown plants. This observation was made with several batches of plants assayed at various times between 3 and 4 weeks after sowing. The difference between the biotypes was not altered much if the flash frequency was varied between 2 and 30 Hz, and it was verified that the intensity of the flashes was saturating (data not shown).

The total number of PSII reaction centers per Chl was also calculated by an independent experimental approach from the number of DCMU-binding sites. Each PSII reaction center has one plastoquinone-binding site to which DCMU binds with high affinity. Although the in vitro electron transport activity of the atrazine-resistant *B. napus* biotype is insensitive to atrazine, it is still sensitive to DCMU (data not shown). Therefore, a DCMU-binding study using $^{14}$C-labeled DCMU according to the method of Tischer and Strotmann (1977) could be undertaken with atrazine-resistant and wild-type thylakoids, although the dissociation constants were different (about 70 and 20 nM, respectively). For the atrazine-susceptible wild type, the number of DCMU-binding sites per mol of Chl in Figure 4B correlates reasonably well with values for mmol of PSII per mol of Chl determined from oxygen flash yields in Figure 4A. For the atrazine-resistant biotype, the number of DCMU-binding sites per mol of Chl was very similar to the wild type at any growth irradiance, in contrast to the 25% lower value for mmol of PSII per mol of Chl calculated from the oxygen flash yields.

We also evaluated the $q_N$ (van Kooten and Snel, 1990) as a function of irradiance. Figure 5A shows that the response of $q_N$ to irradiance was similar in both biotypes grown at 50 $\mu$E $m^{-2}$ $s^{-1}$. When the two biotypes were grown at 350 (Fig. 5B) or 600 (Fig. 5C) $\mu$E $m^{-2}$ $s^{-1}$, $q_N$ responded somewhat differently in the two biotypes: at low actinic irradiances, $q_N$ was higher in the atrazine-resistant biotype than in the wild type, whereas at higher actinic irradiances, the opposite was the case. This "cross-over" effect in high-light plants was observed in several $q_N$ determinations in several batches of plants, and it has also been independently observed recently by van Rensen and co-workers (Curwiel et al., 1993). A high $q_N$ for the atrazine-resistant compared to wild-type PSII at low irradiance would be consistent with an enhanced heat dissipation within the reaction centers containing more $Q_A^-$, favoring nonphotochemical quenching at the expense of photochemical utilization of excitation energy (low $q_P$, see Fig. 1). Based on the cross-over data on $q_N$ for high-light-grown atrazine-resistant versus normal biotypes, we propose that, under low actinic irradiance, measurement of $q_N$ reflects heat dissipation within the reaction center, whereas at high actinic

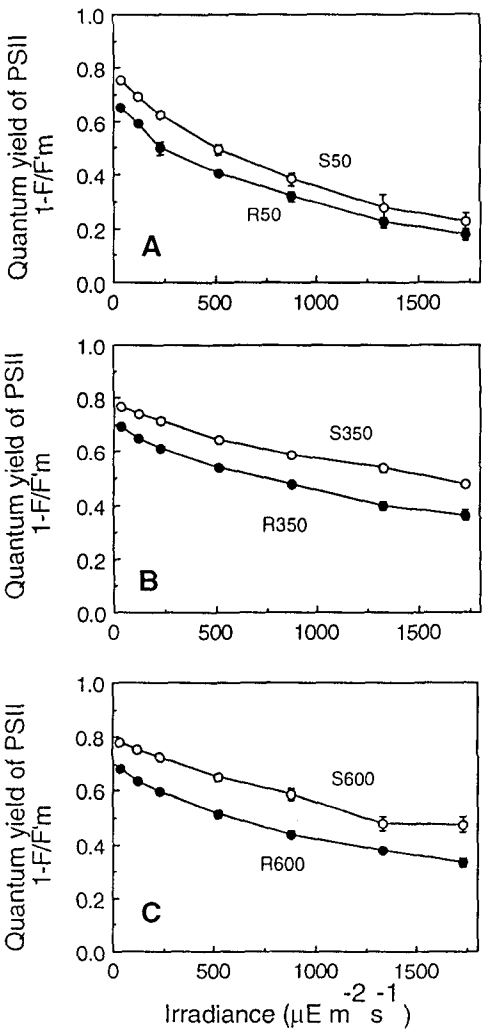


**Figure 3.** The average quantum yield of PSII photochemistry, as expressed by the Chl fluorescence parameter $1 - F/F'_m$ according to the method of Genty et al. (1989), as a function of the incident irradiance on leaves of wild-type (S) or the atrazine-resistant biotype (R) of *B. napus*. The plants were grown under 50 (A), 350 (B), or 700 (C) $\mu$E $m^{-2}$ $s^{-1}$.The values are means ± SE for three or four leaves.

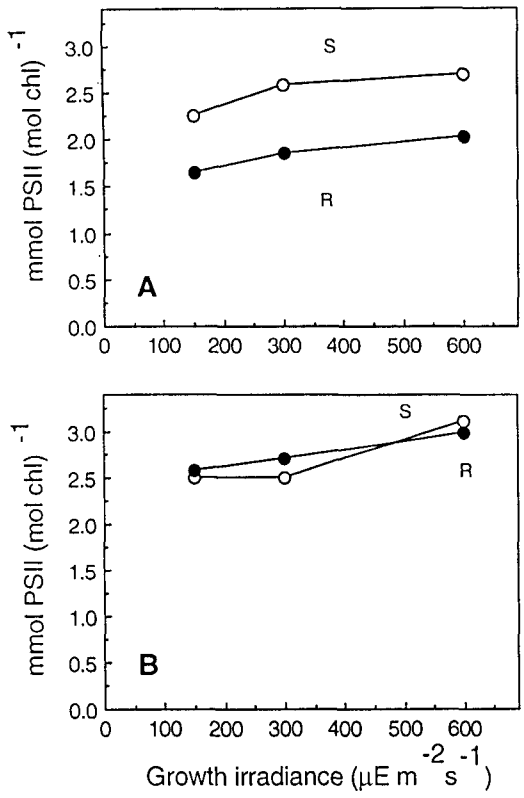


**Figure 4.** The number of functional PSII reaction centers in leaves assayed by oxygen yield during repetitive single-turnover flashes with a flash frequency of 10 Hz (A) and the number of DCMU-binding sites in isolated thylakoids (B), expressed on a Chl basis, as a function of growth irradiance for wild type (S) and the atrazine-resistant biotype (R) of *B. napus*. The data were obtained for three individual leaf discs at each point (A) and for one to two individual preparations of thylakoids from the corresponding plants (B). For each thylakoid preparation, the linear regression was carried out for seven points corresponding to seven concentrations. A comparable difference between S and R in terms of oxygen flash yield (A) was obtained for three batches of plants, irrespective of the day of sampling (between d 19 and 28 after sowing), and in terms of DCMU-binding (B) for two different thylakoid preparations from two batches of plants. The result from one experiment is shown in which the thylakoid preparations used for DCMU-binding were made from the same leaves from which the leaf discs were taken to assay the oxygen flash yield.

irradiances, it reflects heat dissipation within the antennae pigment bed.

Thus, in plant material grown at high, intermediate, and low irradiance, the mutation in which Ser$^{264}$ is replaced by Gly has caused an alteration in PSII in atrazine-resistant biotypes, which is reflected by (a) a slower rate of $Q_A^-$ reoxidation, (b) a larger fraction of closed PSII reaction centers (higher value of $1 - q_P$) during illumination, (c) decreased quantum yields, (d) decreased oxygen flash yields, and (e) increased nonphotochemical quenching at low irradiances. However, no significant differences were seen between atrazine-resistant biotypes and wild types in terms of light-saturated photosynthesis rates of oxygen evolution, expressed on either a Chl or a leaf area basis (Table I), which is consistent with the fact that, at light saturation, in vivo electron transport through PSII is not rate limiting; rather, it is plastoquinol reoxidation by the Cyt *b/f* complex that is rate limiting (Heber et al., 1988).

Thus, under limiting irradiance, the lower quantum yield of oxygen evolution in the atrazine-resistant biotype (Fig. 2) is associated with a decline of PSII photochemical yield estimated from $1 - F/F'_m$ (Fig. 3). In saturating light, the maximal rate of oxygen evolution (and therefore the quantum yield of evolution) are identical in both plant types (Table I), but the PSII photochemical yield remains lower in the atrazine-resistant biotype. The extent of this lower PSII photochemical yield, also corresponding to a lower $q_P$ (Fig. 1), is approximately the same at all irradiances, even at saturating irradiances (Fig. 3). These results may suggest that a fraction of the $Q_A$ pool remains reduced in the atrazine-resistant mutant, whatever the irradiance, and is not involved in the

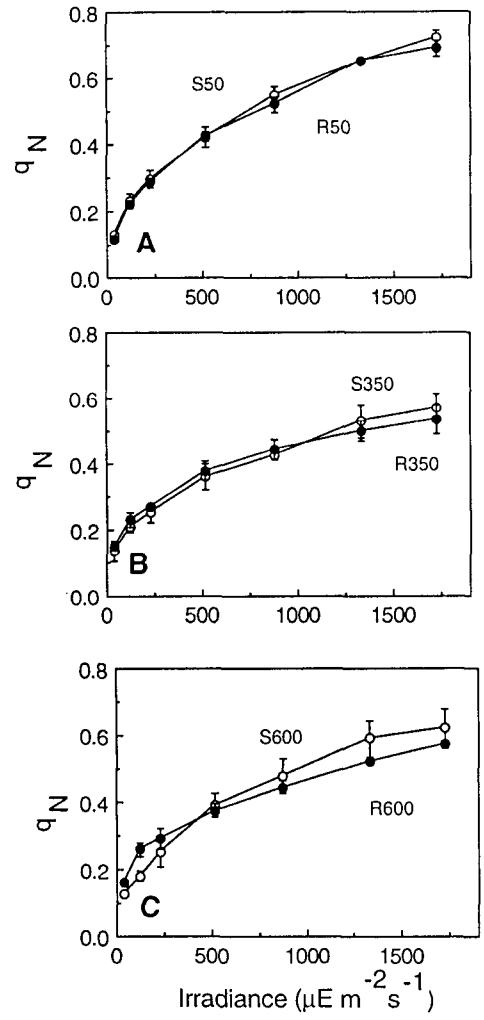


**Figure 5.** $q_N$ measured as a function of irradiance to which leaves of wild type (S) or the atrazine-resistant biotype (R) of *B. napus* were exposed. The plants were grown under 50 (A), 350 (B), or 600 (C) μE m$^{-2}$ s$^{-1}$. The values are means ± SE for three or four leaves.

linear photosynthetic electron transport. These closed centers may well be responsible for the decrease in flash yield in the atrazine-resistant mutant (Fig. 4A).

### Effects of the Mutation on Susceptibility to Photoinhibition

We also studied the susceptibility of the atrazine-resistant biotype to photoinhibition using plants grown at 600 or 50 $\mu$E $m^{-2}$ $s^{-1}$. Leaf discs were exposed to a photoinhibitory illumination of 1600 $\mu$E $m^{-2}$ $s^{-1}$, after which the Chl fluorescence ratio $F_v/F_m$ of leaf discs was determined. This fluorescence ratio is linearly correlated with the number of functional PSII reaction centers (Oquist et al., 1992) and the quantum yields of light-limited oxygen evolution during photoinhibition (Demmig and Björkman, 1987; Hart and Stemler, 1990a; Oquist et al., 1992). Figure 6A shows the decline in the $F_v/F_m$ when leaf discs of high-light-grown *B. napus* plants were photoinhibited for 4 h. The extent of decrease was greater in the atrazine-resistant biotype, confirming the higher susceptibility to photoinhibition for atrazine-resistant biotypes reported by Hart and Stemler (1990b). After 4 h under 100 $\mu$E $m^{-2}$ $s^{-1}$, both biotypes had almost fully recovered.

When grown under 50 $\mu$E $m^{-2}$ $s^{-1}$, plants of both biotypes were much more susceptible to photoinhibition compared to those grown at 600 $\mu$E $m^{-2}$ $s^{-1}$, as indicated by a large decrease in the $F_v/F_m$ ratio, seen already after only 2 h of photoinhibitory light (Fig. 6B). Moreover, for these low-light-grown plants, the greater susceptibility to photoinhibition observed for the atrazine-resistant biotype compared to wild types (Fig. 6A; Hart and Stemler, 1990b) is not so evident. However, the atrazine-resistant biotype showed a poorer ability to regain a high $F_v/F_m$ ratio compared to the wild type.

### Effects of the Mutation on Photosynthetic Acclimation to Growth Irradiance

Atrazine-resistant biotypes are known to exhibit an enrichment of the light harvesting Chl *a/b* complex of PSII and enhanced grana stacking (Vaughn, 1986). Also, with our *Brassica* plants, we could observe from electron micrographs that, at each growth irradiance, the appressed membrane domains were more pronounced in the atrazine-resistant biotype (data not shown). Moreover, for each biotype, the lower the growth irradiance, the more stacked granal membranes were observed, indicating that, although the atrazine-resistant biotype had less-efficient PSII photochemistry at all growth irradiances, it nevertheless retained the ability to acclimate to different growth irradiances. The total number of PSII reaction centers per unit Chl (Fig. 4B) and the maximum photosynthetic capacity of atrazine-resistant biotypes (Table I) increased with growth irradiance, as was also the case for the wild type. Furthermore, the Cyt *b/f* complex content is known to increase with growth irradiance (Wilhelm and Wild, 1984; Evans, 1987; Melis, 1991), and this was also seen for both biotypes; in fact, the enrichment of Cyt *b/f* complex was even more pronounced for the atrazine-resistant biotype (data not shown).

Finally, as an index of plant performance, we determined the fresh weight per plant on d 28 after sowing (Fig. 7). At the two lowest growth irradiances, the atrazine-resistant biotype had a lower fresh weight per plant (87% of the wild type at 50 $\mu$E $m^{-2}$ $s^{-1}$ and 85% of the wild type at 150 $\mu$E $m^{-2}$ $s^{-1}$). At the two higher growth irradiances, the difference

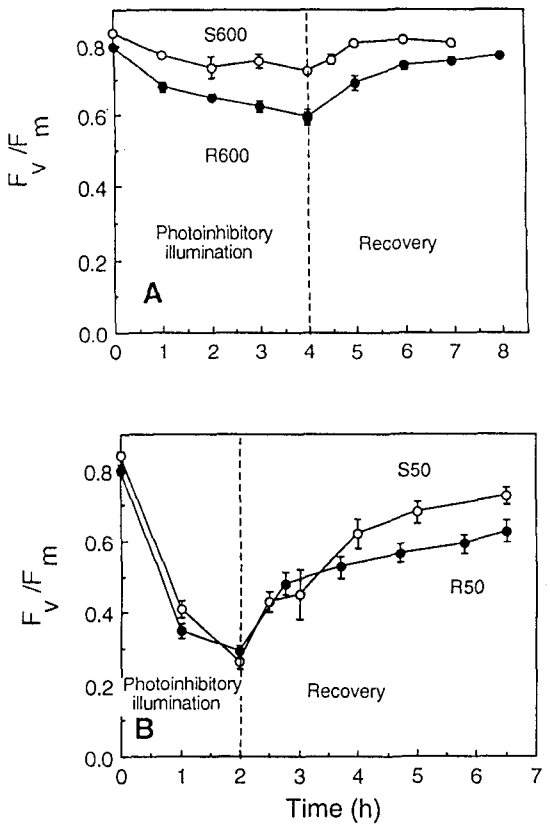


**Figure 6.** The onset of photoinhibition, assayed by the Chl fluorescence ratio $F_v/F_m$, when leaf discs were exposed to an irradiance of 1600 $\mu$E $m^{-2}$ $s^{-1}$ with subsequent recovery under 100 $\mu$E $m^{-2}$ $s^{-1}$. Wild type (S) or the atrazine-resistant biotype (R) of *B. napus*, grown under 600 (A) or 50 (B) $\mu$E $m^{-2}$ $s^{-1}$, were exposed to the photoinhibitory light at 23°C for 4 or 2 h, respectively. The values are means ± SE for five leaf discs.

**Table I.** *Comparison of $P_{max}$, expressed on a leaf area or Chl basis, in leaves of atrazine-resistant (R) and atrazine-susceptible (S) biotypes of B. napus grown at varying irradiance: 50, 350, and 600 $\mu$E $m^{-2}$ $s^{-1}$*

The light-response curves were measured on leaf discs in a leaf-disc electrode containing 21% oxygen and 1% $CO_2$. The values are means ± SE of a number of leaf discs indicated within parentheses.

| Biotype | Growth Irradiance | $P_{max}$ | |
|---|---|---|---|
| | | *$\mu$mol of $O_2$ $m^{-2}$ $s^{-1}$* | *mmol of $O_2$ $mol^{-1}$ Chl $s^{-1}$* |
| S | 50 | 9.1 ± 0.5 (6) | 35.9 ± 4.2 (6) |
| R | 50 | 10.2 ± 0.8 (8) | 34.9 ± 2.3 (8) |
| S | 350 | 37.6 (2) | 74.1 (2) |
| R | 350 | 34.0 (2) | 72.7 (2) |
| S | 600 | 41.6 ± 9.2 (3) | 123 ± 14 (3) |
| R | 600 | 44.3 ± 1.5 (3) | 145 ± 8 (3) |

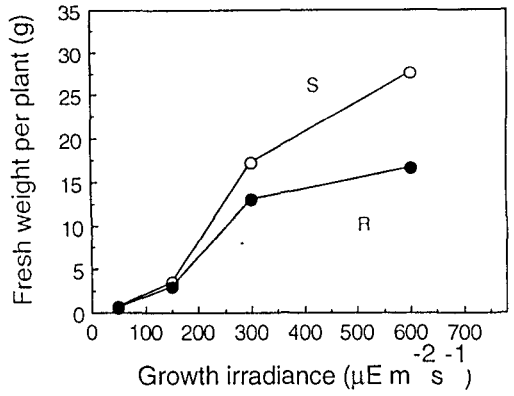


**Figure 7.** The fresh weight per plant as a function of growth irradiance under which wild-type (S) or the atrazine-resistant (R) *B. napus* was grown. Plants were harvested 28 d after sowing.

in fresh weight per plant between the atrazine-resistant biotype and the wild type was more accentuated: the atrazine-resistant biotype performed increasingly less well compared to the wild-type as the growth irradiance increased, yielding 67% of the weight of the wild type at 600 $\mu$E m$^{-2}$ s$^{-1}$.

## DISCUSSION

The primary effect of the mutation, the decreased binding of plastoquinone in the absence of Ser$^{264}$, is reflected by several parameters of changed PSII function that are observed at both high and low growth irradiances: a slower electron transfer from $Q_A^-$ to $Q_B$, a lower $q_P$, lower quantum yield, a lower $1 - F/F'_m$, a lower oxygen flash yield, and a higher $q_N$ at low irradiance. Thus, *Brassica* plants grown under high, intermediate, and low irradiance show an intrinsic impairment of PSII, even without exposure to high light. There is no need to introduce an indirect effect of photoinhibition at high growth irradiance to explain the impairment.

When mutant plants are exposed to high light, secondary effects of the mutation are seen. Rather than being the cause of the lower quantum yield, as suggested by Hart and Stemler (1990b), we propose that the enhanced susceptibility to photoinhibition is a consequence of the mutation, because of the slower rate of electron transfer from $Q_A^-$ to $Q_B$ in the atrazine-resistant biotype.

The longer lifetime of $Q_A^-$ in the atrazine-resistant biotype could increase the probability of generating reactive radicals that can damage membrane components at either the donor or acceptor side of PSII (Barber and Andersson, 1991; Prasil et al., 1992). Recent data based on Chl fluorescence measurements and photoinhibition in vivo suggest that photoinhibition will occur when the reduction state of $Q_A$ is above a threshold corresponding to more than about 40% of the PSII traps being closed ($q_P \leq 0.6$) (Oquist et al., 1992), and if a longer lifetime of $Q_A^-$ would mean that $Q_A$ is more easily reduced beyond that threshold, it could explain why the atrazine-resistant leaves are more easily photoinhibited. Notably, the $q_P$ values were always lower in the atrazine-resistant biotype compared to the wild type (Fig. 1) and in low-light-grown plants, where there is little difference in susceptibility to photoinhibition between the atrazine-resistant mutant and the wild type (Fig. 6A), the $q_P$ values for both biotypes are well past the threshold value of 0.6 as measured at 1600 $\mu$E m$^{-2}$ s$^{-1}$ (Fig. 1A).

However, other factors than increased photodamage due to the altered acceptor-side of PSII also may contribute to the greater susceptibility to photoinhibition in the atrazine-resistant biotype. It could also be related to less photoprotective nonphotochemical quenching via the xanthophyll cycle (Demmig-Adams, 1990), for which there is a slight indication in Figure 5C, or to a limited capacity of the atrazine-resistant biotype to respond to the increased photodamage by increased repair of PSII by D1 protein turnover. In both these cases, one would expect what is indeed experimentally observed, namely, that the difference in susceptibility to photoinhibition between the atrazine-resistant mutant and the wild type is less pronounced in low-light-grown plants (Fig. 6B; van Rensen et al., 1990) and when assayed by photoinhibition in vitro (data not shown; van Rensen et al., 1990; Curwiel et al., 1993). We recently found that the extent of D1 protein turnover in vivo is larger in the atrazine-resistant biotype of *B. napus* than in the wild type under nonphotoinhibitory conditions (Sundby et al., 1993). Under photoinhibitory conditions, the D1 protein degradation seems to occur at already maximal rates in the normal biotype, which would make it difficult for the atrazine-resistant biotype to increase the rate of D1 protein degradation much further (C. Sundby, S. McCaffery, and J.M. Anderson, unpublished data).

The DCMU-binding data we present in this paper (Fig. 4B) show that the lower oxygen yield per flash (Fig. 4A) is due to a lower efficiency of PSII in atrazine-resistant biotypes during repetitive flash illumination rather than to fewer PSII centers on a Chl basis. This lower efficiency of PSII correlates well with the decrease in quantum yield in the mutant (Fig. 2) and the fraction of the $Q_A$ pool that remains reduced at all irradiances (Figs. 1 and 3) and that is not involved in photosynthetic electron transport. The explanation for why a fraction of PSII centers retains reduced $Q_A^-$ is not clear. It can be related either to the slower reoxidation of $Q_A^-$ in the atrazine-resistant biotype, causing some PSII centers to have their $Q_A$ in reduced form when the next flash is given, or to the presence, at all irradiances, of some inactive PSII reaction centers that are incapable of stable charge separation. The half-time determined for the reoxidation of $Q_A^-$ in the atrazine-resistant biotype (3.2 ms) seems to allow significant reoxidation with a dark period of 100 ms between the flashes as shown in Figure 4 and, therefore, exclude the slower reoxidation as an explanation for the lower oxygen flash yield.

On the other hand, the half-time determined after a single flash may overestimate the actual rate of $Q_A^-$ reoxidation in the mutant during repetitive flashes, in which case not only the effect of the altered $Q_B$ binding on the rate of electron transfer from reduced $Q_A^-$ to the bound plastoquinone molecule ($Q_B$) has to be taken into account, but also the exchange of the doubly reduced $Q_BH_2$ with a new plastoquinone molecule. To clarify these events, further investigation of the fluorescence decay during repetitive flashes, as well as of the kinetics of the multiphasic fluorescence decay after a single

turnover flash, is needed. The other possibility is that the atrazine-resistant biotype contains inactive PSII centers; however, the identity of such inactive centers is not obvious. The values of $F_v/F_m$ shown by the mutant was only slightly (3–6%) lower than for the wild type at all growth irradiances (data not shown) but not as low as one would expect if 25% of the PSII centers were photoinhibited during normal growth (e.g. photoinhibition of 40–60% of the functional PSII centers as determined by oxygen flash yield by Sundby et al. [1993]) was associated with a 50–75% decrease in $F_v/F_m$ ratio). However, determinations of $F_v/F_m$ ratios may not be relevant to the status of the whole population of PSII centers in the leaf, and it is not impossible that there could be a limitation in the rate of D1 turnover in the atrazine-resistant mutant, which in combination with an increased rate of continuous PSII damage could adjust the equilibrium in the normal maintenance that keeps the total pool of PSII centers functional by continuous PSII repair, even under nonphotoinhibitory growth irradiances (see below).

Concerning the operation of the mutated reaction center of PSII under saturating irradiances, previous reports are conflicting: lower $P_{max}$ values per leaf area have been reported for *Senecio vulgaris* (Holt et al., 1981; Ireland et al., 1988), *Amaranthus hybridus* (Ort et al., 1983), *Brassica napus* (Jursinic and Pearcy, 1988), and *Amaranthus retroflexus* but not for five other weed species (van Oorschot and van Leeuwen, 1984) or *Chenopodium album* (Jansen et al., 1986). Investigators who have observed a decreased $P_{max}$ usually suggest that this limitation in the maximal photosynthesis rate is the explanation for the poorer performance of atrazine-resistant biotypes in the field. Our data show that, in spite of the decreased efficiency of PSII in light-limited conditions (reflected by reduced quantum yield and lower oxygen flash yield), light-saturated photosynthesis rates were not lowered by the mutation (Table I). However, even if $P_{max}$ is not lower, the reduced crop yields for atrazine-resistant plants under field conditions can still be explained by the effect of the mutation on the PSII efficiency under light-limited conditions, because most crop photosynthesis is carried out under nonsaturating light conditions, at least in temperate regions (Ort and Baker, 1988).

In addition to the intrinsic lowering of PSII efficiency in atrazine-resistant biotypes at all growth irradiances, their enhanced sensitivity to photoinhibition may play a role in reduced crop yields, at least at high growth irradiances, as suggested by Hart and Stemler (1990b). That this is an important factor is indicated by the larger reductions in crop yields of atrazine-resistant biotypes in sunny areas with high irradiances (e.g. Israel) (Gressel and Ben-Sinai, 1985) than in temperate areas. However, there was not much evidence of photoinhibition of either the wild type or the atrazine-resistant biotype during growth under the growth irradiances up to 600 $\mu$E $m^{-2}$ $s^{-1}$ used in this study, as judged by the $F_v/F_m$ ratios, which were approximately 0.78 in the atrazine-resistant biotype compared to approximately 0.81 for the normal biotype, regardless of growth irradiance. Nevertheless, even under conditions in which the plant is not photoinhibited, as judged by $F_v/F_m$ ratios, there is a continuous process of inactivation of individual PSII centers leading to subsequent D1 protein turnover and repair. This is evidenced by the fact that D1 protein turnover is proportional to irradiance (Mattoo et al., 1984) and that photoinhibition takes place faster or at much lower light intensities if D1 protein turnover is prevented by translation inhibitors (Ohad et al., 1984; Lidholm et al., 1987; Tyystjärvi et al., 1992). We have recently found that even under nonphotoinhibitory conditions the extent of D1 protein turnover in vivo is twice as large in the atrazine-resistant biotype of *B. napus* in the wild type (C. Sundby, S. McCaffery, and J.M. Anderson, unpublished findings; Sundby et al., 1993). A larger extent of turnover of the D1 protein may be partly responsible for the slower growth of the atrazine-resistant biotype if it demands energy resources that could otherwise be utilized for growth. Typically, we observed repeatedly in our chloroplast preparations and in electron micrographs that our wild-type plants grown at the highest irradiance always contained large amounts of starch, but plants of the atrazine-resistant biotype had very little. Further study is needed to clarify the extent of this effect on growth and of plant performance.

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